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EPISOMIC TRANSFER BETWEEN SALMONELLA TYPHOSA AND SERRATIA MARCESCENS

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Received March 23, 1961

THE isolation of a culture of *Salmonella typhosa*, strain ST-2, which possessed the unusual characteristic of utilizing lactose (*lac*$^+$) was recently reported (BARON, CAREY and SPILMAN 1959). When this strain was grown in mixed culture with many species and strains of *lac*$^-$ Escherichia, Salmonella and Shigella, it transferred *lac*$^+$ with relatively high efficiency (10^{-3} per ST-2 cell). The recipient cells which received the *lac*$^+$ character were then able to transmit *lac*$^+$ to other *lac*$^-$ strains. Kinetic studies using the blendor technic of WOLLMAN and JACOB (1955) revealed that *lac*$^+$ and donor ability were transferred simultaneously and almost immediately after strain ST-2 and the recipient cells were mixed. Strain ST-2 and hybrids derived from this strain were relatively stable although *lac*$^+$ and donor ability were lost spontaneously at low frequencies. The ability to transfer *lac*$^+$, however, was not eliminated by treatment of ST-2 cells with acridine orange under the conditions described by HIROTA (1960) which were suitable for the removal of the fertility factor, F, from *Escherichia coli*, K-12. Cells receiving *lac*$^+$ and donor ability by conjugation with strain ST-2 did not show the inheritance of any other genetic characters. Likewise, F$^-$ cells of *E. coli* K-12 receiving the characteristics of strain ST-2 did not acquire the ability to transfer their chromosomal markers to other F$^-$ strains. While the *lac* locus injected by strain ST-2 into a recipient may undergo homologous pairing with the chromosome, replication has been found to take place independently of the host's genome (FALKOW and BARON 1961). It is not necessary to assume that the *lac*$^+$ character itself is transmissible, more likely it is carried by an unknown transmission agent probably similar to the F factor of Escherichia. The process observed for strain ST-2 would seem to involve the incorporation of a small segment of genetic material into a transmission agent resulting in a single unit of replication. This genetic element can then be transferred to other strains of bacteria where it may replicate independently of the host genome. The characteristics of strain ST-2, therefore, correspond closely to those of organisms haboring episomic elements (JACOB and WOLLMAN 1958). This class of genetic factors

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are not essential constituents of the cell, and when present, they can behave as an independent unit or be attached to the host chromosome.

While the process observed for strain ST-2 shows similarities to specialized transduction by phage (JACOB and WOLLMAN 1959), conjugation, a characteristic of sexual transfer in bacteria, has been found to be an essential requirement for transfer of the episome. At least two mechanisms act to limit genetic transfer by conjugation. The first of these is differences in mating polarities. The second is divergent structure of genetic material affecting chromosomal pairing and integration. Some of our experiments (to be reported in greater detail elsewhere) have demonstrated that mating polarities essential for the transfer of chromosomal elements in *E. coli* K-12 do not necessarily effect the transfer of the episomic element of strain ST-2. Moreover, the autonomous replication of the episome would seem to overcome some of the difficulty affecting incorporation of the transferred genetic material. It was decided, therefore, to study the possibility of episomic transfer between strain ST-2 and strains of other genera for which there is no positive sign of a common system of mating polarity and which differ appreciably in the base composition of their desoxyribonucleic acid (DNA).

Serratia marcescens has been reported to differ significantly from Salmonella, Escherichia and Shigella in the base composition of its DNA (BELOZERSKY and SPIRIN 1960). In addition, repeated attempts to demonstrate chromosomal transfer between Hfr and F^+ Escherichia or Salmonella and Serratia have been unsuccessful in this laboratory. We therefore, selected this species to test with strain ST-2.

A stable *lac*$^-$ prototrophic strain of *S. marcescens*, SM-6, was grown in mixed culture with strain ST-2. Since strain ST-2 requires cystine and tryptophan for growth, minimal lactose agar was used to detect hybrids. *lac*$^+$ progeny, otherwise identifiable as Serratia, were observed at the low frequency of 4×10^{-8} per donor cell. Matings between strain ST-2 and *lac*$^-$ *S. marcescens*, strains USC, and A-12 (nonpigmented) gave essentially the same results.

While bacterial DNA preparations have proved to be highly homogeneous in base composition, nevertheless, measurable compositional heterogeneity does occur (MARMUR and DOTY 1959). In order to be certain that the Serratia strains used in our experiments did, indeed, differ significantly from strain ST-2, we determined their guanine-cytosine (GC) content by estimation of the thermal denaturation temperature of their DNA (MARMUR and DOTY 1959). The GC content of these Serratia strains was found to be 58 percent as compared to a figure of 50 percent for strain ST-2 and *E. coli* K-12. These estimations are in good agreement with those previously reported in the literature (MARMUR and DOTY 1959; BELOZERSKY and SPIRIN 1960).

In order to simplify the selection and further purification of the Serratia hybrids, a number of streptomycin resistant mutants of strain SM-6 were isolated by plating dense suspensions of cells on nutrient agar containing 600 μg/ml of streptomycin. Some of these streptomycin resistant (S^r) clones showed slight increases in fertility with strain ST-2, while one clone, SM-6-S^r-11, mated at a

frequency of 10^{-4} per ST-2 cell. This clone was unchanged in GC content and still failed to yield progeny when plated with F+ and Hfr cultures of *E. coli*.

lac+ hybrids of Serratia were picked and tested for their ability to transfer the *lac*+ determinant to *lac*− strains of Salmonella, Shigella and Escherichia. All of the hybrids tested were able to transfer *lac*+ although the frequencies observed in these experiments varied considerably. Some of the Serratia progeny acted as donors only at low frequency (10^{-8} per donor cell), but the majority were competent donors at a frequency of about 10^{-5}. A summary of the mating experiments is shown in Table 1.

These results should be considered in view of the concept developed recently (LANNI 1960; MARMUR and SHILDKRAUT and DOTY, in preparation) regarding genetic homology in relation to the DNA base ratios of the microorganisms involved. In all instances of genetic transfer between bacteria established to date, the organisms involved have similar DNA base ratios. This infers that strains differing appreciably in their DNA base ratios should not be genetically homologous. While this hypothesis may hold true for the integration of chromosomal DNA, our data indicate that small, autonomous units of genetic material can be transferred between organisms with dissimilar DNA base ratios. This observation has been extended with organisms carrying other episomic elements. We have observed that a strain of *E. coli* harboring an episome, F-*lac*, consisting of the fertility factor and *lac* locus (JACOB and ADELBERG 1959) and a strain of Salmonella carrying an episome conferring resistance to several antibiotics (MITSUHASHI, HARADA and HASHIMOTO 1960) can transfer their episomic elements to Serratia.

The divergence between the DNA base ratios of the original strains carrying episomes and Serratia led us to consider the possible demonstration of molecular heterogeneity in DNA prepared from Serratia hybrids. Using the cesium chloride gradient technic (MESELSON, STAHL and VINOGRAD 1957) it was found that the DNA isolated from the Serratia and Salmonella parental cultures had different buoyant densities estimated from their banding positions. In contrast, the DNA isolated from the *lac*+ Serratia hybrids gave rise to two bands in the analytical ultracentrifuge, one corresponding to the density observed for the

TABLE 1

Episomic transfer between Salmonella typhosa, *ST-2 and* Serratia marcescens

lac− recipient organism	Percent	Frequency of hybrid formation selection for *lac*+ with		
		S. typhosa ST-2 (50%)	*E. coli* W1895 (Hfr) (50%)	Serratia* SM-6 *lac*+ (58%)
Shigella dysenteriae	(50)†	5×10^{-3}	1×10^{-4}	1×10^{-5}
Salmonella typhimurium LT-7	(50)	2×10^{-3}	1×10^{-7}	2×10^{-5}
Escherichia coli K-12	(50)	1×10^{-3}	1×10^{-3}	2×10^{-5}
Serratia marcescens SM-6	(58)	4×10^{-8}	0	.....
S. marcescens SM-6-S^{r}-11	(58)	1×10^{-4}	0	5×10^{-5}

* Derived from a cross with strain ST-2.
† Percentage figures correspond to the guanine-cytosine content of the organisms.

parental Serratia and another, comprising about one percent of the total DNA, corresponding to the density observed for the Salmonella parent (MARMUR, ROWND, FALKOW, BARON, SCHILDKRAUT and DOTY 1961). At present, we assume that the small amount of DNA with the buoyant density of the Salmonella parent represents the transferred episome.

SUMMARY

The transfer of lactose utilization and donor ability from *Salmonella typhosa*, strain ST-2, to *Serratia marcescens* via an episomic mechanism is described. This transfer is of particular significance because of the divergent DNA base ratios of the parental strains and the lack of a demonstrable common system of mating polarity.

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A TEST FOR SOMATIC MUTATION IN THE ORIGINATION OF CONVERSION-TYPE INHERITANCE AT THE B LOCUS IN MAIZE[1]

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Received April 21, 1961

THE original two maize plants for which a conversion-type inheritance pattern has been described (COE 1959) arose in a uniform intense line that continues to give rise to new exceptions at variable frequency. Because of its significance to an understanding of the mechanism of the instability, the question arises: Does the mutational change from orthodox to conversion-type inheritance in this line occur at all stages in the life cycle or is it restricted, for example, to meiosis?

In plants of the proper genotype it might be possible to observe directly for somatic sectors representing premeiotic changes, but the sun-red background of the original line precluded this simple determination. However, the special structure of the corn ear, which permits the ordered planting of seeds according to the ordered development of the inflorescence, provides the opportunity for such a test in ear diagrams, since each kernel represents a sample of the cell lineage of itself and its neighbors.

The ear-diagram method apparently was first used in maize by ANDERSON (1923) in a study of maternally inherited chlorophyll patterns. The familiar demonstration of somatic variegation and development of sectors for pericarp color (maternal tissue) constitutes an ear diagram test without the requirement of planting to observe the phenotype.

For the present purpose, ears from crosses of unstable *B* egg parents by stable *B* pollen parents were used. The area of an ear to be tested was selected and the kernels were numbered with a pencil. Skips in the pattern were noted and the kernels were planted in order, in standard serpentine fashion. The resulting plants were graded on a scale of increasing intensity of plant color, from 0 to 6. Subsequent progeny tests have confirmed that the apparent demarcation between *B′* mutants (grade 0, 1, or 2) and *B* (grade 3, 4, 5, or 6) is real; the grades have been reduced to mutant and nonmutant in the following.

Sectoring was not obvious in the resulting figures; a numerical index therefore was developed to express the degree of association (non random distribution) of the mutants. The reasoning used, similar to that of SPRAGUE (1935) in his

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analysis of the randomness of segregating phenotypes, was as follows: If there is no sectoring, the probability that a selected B' individual (the "mutant" type) will have another B' individual adjacent to it in a given direction will be the frequency at which B' individuals occur in the remainder of the area studied. Similarly, the probability for an unlike neighbor will be the frequency of unlike individuals in the remainder of the area. These expected frequencies of mutant-mutant and mutant-normal pairs can be subtracted from the observed frequencies determined by counting the frequency of adjacent B', adjacent B, and adjacent skips (as a counting check), for each B' individual in each direction, and will leave a residual excess or deficiency of like and unlike pair frequency. The difference between the two residues (B'–B' minus B'–B) will serve as an indication of the amount of non randomness, with indexes ranging from approximately -1.00 to $+1.00$ or higher. To test the index as a measure of sectoring, eight hypothetical diagrams of five rows by ten kernels were drawn, or generated by coin tossing. These diagrams, with the individual index values, are presented in Figure 1; dots represent the mutant individuals in an array with unmarked normals. The first five diagrams in the figure were drawn arbitrarily, the sixth and seventh (indexes -0.02 and $+0.28$) were generated by coin tossing, and the last was generated by coin tossing within a central three- by six-kernel area (representing a sector segregating for mutant). The efficiency of the index can be seen in the numerical expression of the degree of association in these diagrams; Table 1 shows the tabulation and calculation of the index for the diagram with a value of 0.62. No direct statistical analysis was attempted.

Of the several actual ear diagrams studied, only six included both mutant and normal individuals. In Figure 2 the mutants are represented by dots, as in Figure 1, and skips by dashes. Five of these diagrams are of five-row by ten-kernel areas; the other includes the majority of a 14-row by 18-kernel cylinder. More than 40 of the individuals in the larger diagram were progeny tested and confirmed, with minor exceptions, for the grades given them as individuals.

An entirely random pattern to the origination of new B' individuals is evident. The mutation is not a simple segregational event, of course, since the frequency of B' individuals in various progenies varies over the entire range from zero to 100 percent.

On the basis of these observations it can be concluded that the mutational event in the change from orthodox to conversion-type inheritance occurs (at least

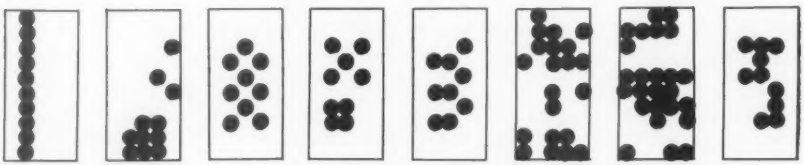

FIGURE 1.—Hypothetical ear diagrams and association indexes (See text for details.)

TABLE 1

Method of calculation of association index

		Number of occurrences: Mutant-mutant	Mutant-normal	Total	
Below		5	3	8	
Above		5	6	11	
Left		5	6	11	
Right		5	4	9	
	Observed no.	20	19	39	
	Expected	10/49	39/49		
	Observed freq.	0.513	0.487		
	Expected freq.	0.204	0.796		
	Difference	+0.309	−0.309		
	Index	+0.618			$= (f_{mm} - p_{mm}) - (f_{mn} - p_{mn})$

primarily) between the initiation of ovule primordia and embryo differentiation. If the event is restricted to this period, then it most probably occurs during meiosis, and its mechanism is probably chromosomal or genic rather than cytoplasmic or infectious.

The potential for this mutational change cannot have existed indefinitely in the evolutionary history of maize; else unchanged *B* could hardly have persisted to the present. There is no evidence for reversion of *B′* to *B*, or, more particularly, to stable or intractable *B*. If the basis for this potential is a simple error in a normal cellular mechanism, for example differentiation, this must be a very rare type of error; a natural population of cross-fertilizing organisms could not ordinarily withstand sudden sweeping dispersion of mutant types and might be expected to have selected systems that ordinarily circumvent their occurrence.

SUMMARY

By the use of ear diagrams and a numerical index to express degrees of non randomness, a test was conducted for premeiotic sectoring in the change from

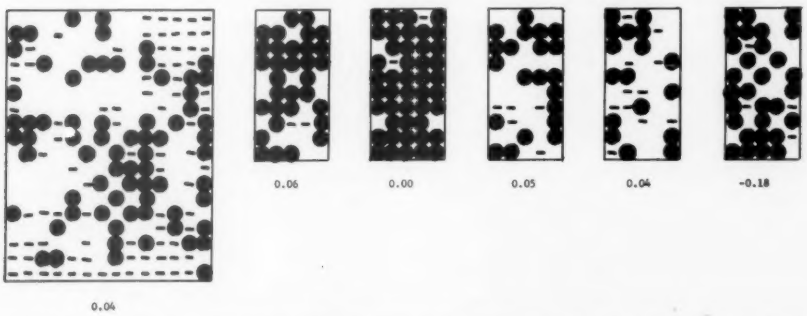

FIGURE 2.—Ear diagrams and association indexes for progenies giving rise to conversion-type inheritance.

orthodox to conversion-type inheritance of plant color at the *B* locus in maize. Sectoring was not detected; a meiotic (chromosomal or genic) mechanism is suggested to be responsible for the mutational event.

It is pointed out that the effects in a population of instability systems like that giving rise to *B′* would be so sweeping that selection might favor mechanisms circumventing this type of error.

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THE REGULAR REINVERSION OF THE ROUGHEST^3 INVERSION

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Received August 9, 1960

PRECISE reinversions of inverted sequences of *Drosophila melanogaster* have been reported by GERSHENSON (1934) and by GRUNEBERG (1936, 1937). These accounts were subsequently questioned by others (STURTEVANT and BEADLE 1936; KAUFMANN 1942) and in general greeted with some skepticism since there did not appear to be any basis for the implication of a greater breakability at the break points of an inverted sequence than at any other point along the chromosome length. A mechanism that would account for such an apparent greater "breakability" was suggested by NOVITSKI (1946). This hypothesis was inspired by a consideration of the concentration of inverted sequences in element C of *Drosophila athabasca* and *D. pseudoobscura* and by the non random distribution of the break points of those inversions, particularly when compared with the breaks of pre-existing inversions more primitive in the phylogenetic sequence. It proposed simply that in the classical prophase configuration of an inversion heterozygote, in the regions of the break points where asynapsis must necessarily occur, the unsynapsed strands are regularly to be found in unusually close proximity, and should breakage of these strands occur (whether naturally, or caused by a "strain" produced by the synaptic force, or caused by a propitiously placed single ionization which would break both strands, being for the purposes of this argument irrelevant) the broken ends being very close to each other would more readily than usual unite to form a new sequence. In this way the break points of the newly arisen rearrangement would be determined by the synaptic configuration of the two participating chromosomes. Under these conditions reinversion might occur with a very much higher frequency than would be expected on a simple breakage-fusion hypothesis.

EXPERIMENTAL PROCEDURE

Even if the conditions outlined above hold and cause a manyfold increase in the spontaneous production of new gene sequences, the absolute frequency might be so low as to make their experimental detection by usual crossover suppression tests impracticable. For this reason, along with the observation of GRUNEBERG which suggested that reinversion of *rst*^{3} (roughest, X chromosome, 1.7) could occur and be detected readily by disappearance of its associated position effect, that inversion was selected for study. The event was measured in the progeny of females heterozygous for the *rst*^{3} inversion and a normal chromosome, as the hypothesis demands. The parental females were lightly X-rayed with 1800r units.

In order to exclude any possibility of rare double crossovers between the normal and the *rst*^{3} chromosomes which might simulate reinversions, the normal X chromosome was marked with the mutants *y* (yellow, 0.0), *sc* (scute, 0+), w^{col} (colored, 1.5), *spl* (split, 3.0) and *f* (forked, 56.7), and the *rst*^{3} inversion with *v* (vermilion, 33.0), and *f*. The mutant vermilion is removed from the *rst*^{3} chromosome rather readily by double crossing over and so adds little weight to any of the arguments presented below.

Figure 1 shows the relative position of the loci used to mark the normal and inverted sequences. Except for forked, which is homozygous, it is implied that each normal allele is to be found on the homolog at a point opposite the position of each recessive allele. Figure 1a shows how two strands may cross each other in an inversion configuration. Figure 1b is the same configuration drawn differently in order to indicate most simply the types of exchanges (A, B and C) that are relevant to a discussion of reinversion. In both cases, the distances between the white, roughest, and split loci are greatly exaggerated for the sake of clarity; for all practical purposes these may more reasonably be considered adjacent loci.

After the irradiation of virgin females of the constitution described, several were put into each culture bottle along with an equal number of Canton-S males. At two successive intervals of seven days the adults were transferred to new bottles. Male progeny only were scored. Since both *rst*^{3} and *spl* are characterized by a roughened eye, reversions of the phenotype, with a normal smooth eye, are easily detectable. However, the *spl* allele marking the normal chromosome is readily distinguishable from *rst*^{3}, and all male progeny were classified according to which they carried.

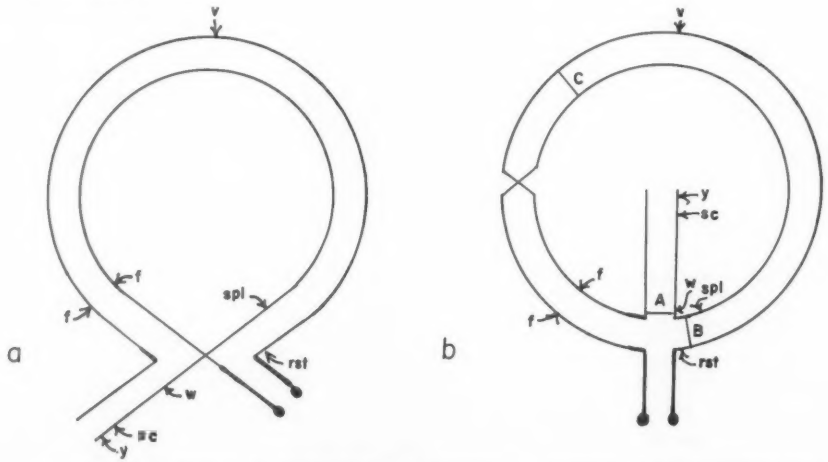

FIGURE 1.—a. Diagram of configuration of heterozygous inversion, *rst*^{3}/+, emphasizing region of crossing of strands; b. Inversion configuration, *rst*^{3}/+, drawn so as to indicate most simply position of crossovers necessary to simulate reinversion.

TABLE 1

The number of reversions of rst^3 *over the total* rst^3, *found in the progeny of heterozygous females, classified by transfer number for each of four series*

	Series			
	A	B	C	D
Original	0/357	0/508	3/7,466	2/4,203
Transfer 1.	0/120	1/321	1/5,199	1/5,209
Transfer 2.	0/1,264	0/471	2/3,243	1/3,412

RESULTS

Table 1 shows the number of reversions over the total *rst^3* chromosomes recovered in the male progeny. Not given in the table are the crossovers between the *y* and *w* loci, of which there were 15 altogether, and certain other irregular types to be discussed later. The reversions, numbered arbitrarily, are described individually below.

1. *rst^{3+2}* (from set B, first transfer). Phenotype: *v f*, *rst^3* completely suppressed. Salivaries: normal sequence. Crossing over: normal, when over normal sequence.
2. *rst^{3+6}* (from set C, original culture). Phenotype: incomplete suppression of *rst^3*; wings plexate; suppresses Notch and exaggerates Confluens. Salivaries: *rst^3* sequence, with small duplication in vicinity of *rst* locus. Crossing over: normal when over *rst^3* sequence.
3. *rst^{3+7}* (from set C, original culture). Phenotype: complete suppression of *rst^3*, otherwise parallels preceding case. Salivaries: unaltered *rst^3* inversion, although possibility of small insertion as in preceding case not excluded. From different bottle than *rst^{3+6}*, therefore of independent origin.
4. *rst^{3+8}* (from set C, original culture). Phenotype: wings strongly plexate; homozygous female poorly viable, and infertile; *rst^3* completely suppressed. Salivaries: normal sequence with small duplication of undetermined origin and constitution at *rst* locus. Crossing over: about 50 percent normal.
5. *rst^{3+9}* (from set D, original culture). Phenotype: complete suppression of *rst^3*; no other phenotypic effect. Salivaries: not examined, see below. Crossing over: normal, when over normal sequence.
6. *rst^{3+13}* (from set D, original culture). Phenotype: complete suppression of *rst^3*. Genetic tests show dominant suppressor on chromosome 3 (does not suppress *rst^2* or *w*).
7. *rst^{3+15}* (from set C, first transfer). Phenotype: *rst^3* only partially suppressed. Salivaries: *rst^3* sequence unaltered. Crossing over: normal over *rst^3* inversion.
8. *rst^{3+17}* (from set C, second transfer). Similar to *rst^{3+2}*.
9. *rst^{3+88}* (from set C, second transfer). Similar to *rst^{3+2}*.
10. *rst^{3+125}* (from set D, first transfer). Similar to *rst^{3+2}*.
11. *rst^{3+204}* (from set D, second transfer). Similar to *rst^{3+2}*.

The 11 cases listed above represent all those in which the *rst^3* position effect

had disappeared (completely or in part), leaving *v* and *f* intact. In the preliminary analysis, given above, six of these seemed to have a sequence inseparable from normal either by the usual crossover tests or by salivary examination. For this reason, these chromosomes were subjected to a very much more sensitive test. The attention of the reader is directed to the following description of that test because it is far more revealing than an ordinary crossover test and would uncover quite small discrepancies between a normal and reinverted chromosome if they should exist; it should further be emphasized that the resolution of this technique is far beyond that of any common cytological method and has made the application of such methods unnecessary. The procedure is as follows: If the chromosomes were not precise reinversions, then the resulting sequence would be quite like a normal one, except that there would remain in a distal position a section of chromosomes normally proximally placed, or *vice versa*, or both. If such a chromosome were heterozygous with a normal chromosome, a single exchange located anywhere in the region between the break points of the original inversion would produce crossover chromosomes with duplications, or deficiencies, or both. These six chromosomes were therefore placed against normal X chromosomes, multiply marked, and complementary single crossover products were tested in males and as homozygotes in females to determine if there might be any phenotypic effects of these derived chromosomes. Furthermore, males carrying these crossover chromosomes were mated to attached-X females lacking a Y chromosome; the absence of the *bb*, nucleolus organizing, or any other essential region would be manifest by an absence of F_1 males. In none of these cases was there any evidence of anything other than a completely normal chromosome; these six cases, then, are considered to be reinversions.

Not listed in the table are other exceptional types. There were a total of 11 cases of crossing over between the *sc* and w^{col} loci; six cases where the males were *spl*, not w^{col}, and one case where they were w^{col} and not *spl*. The latter seven have been analyzed to a limited extent; they are not crossovers but appear to be roughly analogous to changes on the normal chromosome corresponding to the five miscellaneous changes on the rst^3 chromosome, in that a few involve duplications show phenotypic effects of duplicated material and suppress crossing over whenever with a normal or rst^3 chromosome. The chromosome carrying w^{col} but lacking *spl* appeared cytologically to carry a normal sequence; crossing over between it and a normal chromosome was essentially normal. One of the contrary types (*spl*, non-w^{col}) appeared to carry the rst^3 sequence, with a small duplication at the rst^3 break point, in agreement with the genetic observation that crossing over is freer when over rst^3 than when over a normal chromosome, although not completely free in the first instance. A second case of this phenotype showed a normal sequence cytologically and a third case, not examined cytologically, gave normal crossover values with a normal sequence, except near the tip of the chromosome, once again indicating the presence of a duplication.

Even more interesting is another frequent type, in fact, the most frequent type in the entire experiment. These are phenotypically forked, with none of the other

mutants. Unlike the *v f* reversions described above, these are found in the later transfers; never in the original culture, and when a few appear in the first transfer of a culture, a large cluster is usually found in second transfer, indicating a mitotic, rather than meiotic, origin. There was a total of 11 such clusters, including in all 164 individuals. Usually these males were sterile; however five such males were fertile and so could be tested further. In these cases, the salivary chromosomes were normal, and the tests described previously, of fractionating the X chromosome to test for male compensating duplication deficiencies, gave results similar to those reported for the *v f* reversions; i.e., there was no difference between these sequences and a normal one. Subsequent attempts to repeat this observation, both with the original stocks used in the experiments reported here and with differently marked stocks, have been unsuccessful.

DISCUSSION

In defense of the thesis that the six reversions described here are precise reinversions, two alternatives must be examined critically. The first of these is that these cases are nothing more than crossovers in which the alleles marking the normal chromosome (*y*, *sc*, w^{col}, *spl*, v^+) have been replaced, by crossing over, by the alleles from the rst^3 chromosome. Now, such an event would have to take place by a triple crossover; first, a single outside the inversion, but immediately adjacent to the break point of the rst^3 inversion, (crossover A in Figure 1b) which would replace *y*, *sc*, and w^{col} by the normal alleles found on the rst^3 chromosome, and, second, a double inside the inversion, one taking place again immediately adjacent to the break point of the inversion (crossover B in Figure 1b) and the other on the side of the vermilion locus (crossover C in Figure 1b). The first event, the crossover (A) adjacent to break point and outside the inversion but occurring by itself and represented by the phenotypic class y^+ sc^+ w^+ *spl* v^+ *f* (the complementary type not having been detected) was found in six cases, but of three analyzed, two were not ordinary crossovers but were rather gross alterations of chromosome structure easily demonstrated cytologically. In any case, a rough, and undoubtedly exaggerated estimate of the frequency of this crossover would be 6/32,000.

As for the second event (the frequency of doubles within the inversion—crossovers B and C), the type demanded by the crossover explanation of the reversions would give rise to the phenotypic classes *y sc* w^{col} rst^+ spl^+ *v f* and y^+ sc^+ w^+ rst^3 *spl* (*vnu*$^+$) *f*. None of the second type were found, perhaps because they were indistinguishable; but the first type, which would be unambiguous and not easily overlooked, was found once. The frequency of this double crossover is of the order of 1/32,000. Not only would one not expect 6/32,000 cases where both the single crossovers outside and the double crossovers inside the inversion occurred simultaneously, but taking into account the further point that for extremely close loci as w^{col} and *spl* are, one might expect coincidence to diminish the expected frequency of doubles even more.

The second possibility which must be considered is that the "reinversions"

result from nothing more than random breaks along the chromosome length, with subsequent refusions. In this case, selection by reversion of the phenotype would necessarily limit those cases saved to that group which has suffered a break between the *rst* locus and the heterochromatin, with subsequent separation of the two. If we review the characteristics of the 11 reversions, we see that, one reversion, the dominant suppression on the third chromosome, is irrelevant to the argument, three others appear to have small insertional duplications at the *rst* locus (with an effect not only on rst^3, but also on Notch and Confluens) and one (rst^{3+15}), a partial reversion, appears to have involved no structural change. The other six are the "precise" reinversions. Thus we have no case where the second break in the chromosome appears at any point other than the original break point, as far as can be determined. Yet it has been shown beyond any doubt by KAUFMANN (1942) that rearrangements involving second breaks in many other parts of the genomes can also give rise to reversion of the phenotype. Even if one discounts the likelihood of breaks in the autosomes participating in rearrangements to revert the *rst* phenotype, because of the relatively infrequent production of *bona fide* translocations after irradiation of female Drosophila, there would be still ample opportunity for the recovery of reversions with both breaks in the X chromosome. It should be noted that in the experiments of KAUFMANN, where males were irradiated, six of 16 reversions analyzed involved two breaks in the X chromosome (ten of the 16 were reciprocal or insertional translocations), and each of the six could easily be shown not to be "reversions." This is quite different from the present case, where none of the reversions from females are like those described above, and six appear to be exact reinversions. A reasonable conclusion is that the two kinds of experiments give quite different results.

The conclusion, then, is that the heterozygous inversion configuration facilitates the production of new sequences and, in particular, reinversions. It should be noted, however, that the reinversion of rst^3 originally reported by GRUNEBERG came from a homozygous, not heterozygous, stock. It is possible that the homozygous sequence forms a pseudoloop as the distal heterochromatin near the white locus in the inversion pairs with proximal heterochromatin, just as it does in salivary gland chromosome preparations. In this way conditions which would facilitate reinversion would still exist.

The study of naturally occurring sequences in certain species (*Drosophila pseudoobscura*, *D. athabasca*) has brought up two puzzling questions: (1) Why is the variability of one chromosome (element C) so much more widespread and frequent than that of the others? and (2) Why does there appear to be a non random distribution of the break points in the various sequences of the variable chromosomes? A new insight into the first question has been provided by the work of TERZAGHI and KNAPP (1960), which suggests that in *D. pseudoobscura* heterozygosity for inversions in more than one chromosome is responsible for sizable egg mortality (presumably because of non-homologous pairing followed by irregular disjunction) and that, therefore, only one chromosome may show

widespread variability without adversely affecting the fertility of the species. The second puzzle may be answered by the present work.

It should be noted that in recent years, more instances of this sort have been uncovered. A non random distribution of break points of inverted sequences found in natural populations has been reported for *Drosophila subobscura* (GOLDSCHMIDT 1956a,b) and certain chironomids and black flies (ACTON 1955; ROTHFELS and FAIRLIE 1957; BASRUR 1959; DUNBAR 1959). ROTHFELS and FAIRLIE (1957) have reviewed the evidence for this type of non randomness in detail, showing, for instance, that in *Drosophila melanica* also there appears to be a similar situation when the descriptions of inverted sequences by WARD (1952) are examined closely.

SUMMARY

Females heterozygous for the roughest[3] inversion were X-rayed, and their progeny examined for reversions of the phenotype. Six were found which, by both genetic tests and cytological examination, appeared to be precise reinversions of the roughest[3] inversion. It is suggested that the inversion loop formed in the heterozygote facilitates breakage and reunion in the vicinity of the break points of the original inversion.

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SOME OBSERVATIONS BEARING ON PLASMID VERSUS GENE HYPOTHESES FOR A CONVERSION-TYPE PHENOMENON[1]

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IN a recent paper, ALTENBURG (1959) has suggested an explanation, based on a complex but feasible interaction between the gene and a virus, for the paramutation phenomenon discovered by BRINK (1956) at the *R* locus in maize. This suggestion, stated in simple form, postulates a gene-maintained particle that would survive in the cell as long as the nucleus contains the proper genic factor(s) but would disappear when a necessary factor is missing. An improbably complex set of assumptions and conditions is required in order to ascribe the paramutation phenomenon to such a system, particularly in view of the specific relationship such particles would need to have with particular alleles (BRINK, BROWN, KERMICLE and WEYERS 1960). Applied to the simple, regular phenomenon at the *B* locus in maize, reported by COE (1959a), the particle hypothesis would seem to be fully as tenable as any hypothesis of gene-gene interactions might in our present state of knowledge and has distinct merit as a concrete mechanism with definable limitations. The present paper deals with some observations bearing on the applicability of the ALTENBURG hypothesis to the *B′* case. As yet a definitive test has not been carried out.

MATERIALS AND OBSERVATIONS

In the present experiments a stock of *B′* (converter; weak sun red plant color) derived from one of the original two cases of *B′* through a repeated series of crosses to intense (*B*) was used. This stock has continuously given uniform *B′* progenies, as previously described (COE 1959a), and was used in the crosses to *b* (green) which have served to demonstrate that the converter property segregates in parallel with segregation at the *B* locus. The marker stocks for chromosome 2, involving various combinations of *lg* (position 11), gl_2 (30), *b* (49), *sk* (56), *fl* (68), and v_4 (83), were kindly supplied by E. B. PATTERSON from the Maize Genetics Cooperative stocks. Marked stocks of *B*, *b*, B^b, and B^e were used. The last two of these alleles apparently have not been described previously in the formal literature and are additions to the allelic series of *B*, B^w, and *b* (respec-

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tively, intense, weak, and no anthocyanin pigment in the husks, glumes, and other plant parts) described by EMERSON (1921).

B^b (barred glumes) is associated with very weak or no pigmentation in all parts except for an intensely colored bar at the base of the tassel glumes. In the absence of authentic stocks of B^{wc} it is not certain that B^b is different from B^{wc}, but B^b plants are much less strongly pigmented than the B^{wc} plants described and pictured by EMERSON. B^b and B' plants are so closely similar that in at least some backgrounds they are phenotypically indistinguishable.

B^v (variegated) was discovered by W. R. SINGLETON (in STADLER's laboratory) in a progeny from ultraviolet-irradiated B pollen; current usage would apply the symbol b^m (mutable b) because the plants are green (b) except for scattered, clearly defined sectors of variable size and intense (B) color. Germinal changes of B^v to stable B are found at a fairly high frequency (ten in 509 in a test by the writer), and the control of mutability seems to reside at the B locus itself.

Dominance relations of B, B^b, B^v, and b are just as might be predicted on the simplest assumptions: B is dominant over the others; B^b and B^v are dominant to b; B^b and B^v are both expressed when present together, in those tissues where both can be observed.

Stocks of B^v were obtained from M. G. NUFFER; B^b was present as "b" in certain of the chromosome-2 marker stocks and is rather widespread among genetic lines in general.

Normal segregation and recombination of markers flanking B′: A test of segregation of markers on each side of the B locus in conversion heterozygotes (B'/B) has dispelled any remaining doubts concerning transmission anomalies (see COE 1959a) that might have explained the conversion-type phenomenon. A consequence of the postulated anomalies would be lowered transmission of markers associated with B in B'/B heterozygotes. Only gl_2 and *sk*, the two closest markers to B, need be accounted for here; *lg* and v_1 were included in the test and gave confirmatory results.

Paired hybrids of $+\ B'\ +$ and $+\ B\ +$ with *gl B sk* were self-pollinated and backcrossed to *gl B sk*. The B' test and B comparator members of the pairs were as closely alike as possible in background derivation. The progenies were classified for the markers and graded for plant color on a scale ranging from 0 (color in the glume bar only) to 5 (full intense). Within limits set by the modest populations used, the markers showed normal recombination, segregation, and transmission (Table 1). In the B comparator self the few overlaps in grade can be attributed to the occurrence of new B' exceptions in this background.

Intractability of an intermediate allele (B^b): The property of b that has implicated the B locus in the phenomenon under discussion is its intractability to B' in B'/b heterozygotes. Since b is functionally null, it can be considered provisionally as a deficiency or the equivalent, lacking the necessary structure or function for the accomplishment of the conversion event. B^b, though similar to b, has the capacity to function as B to a slight extent, and its tractability is therefore of interest. Paired hybrids of $+\ B'\ +$ and $+\ B\ +$ with *gl* B^b *sk* were self-pollinated

TABLE 1

Color-grade distributions and Gl_2-Sk *recombination in marked self and backcross progenies of* B′/B *and* B/B *comparator*

	Markers	+ +						+ *sk*						*gl* +						*gl sk*							
	Grade	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5	Sum	Rec. pct.
+ *B′* + / *gl B sk*	self	34	4	1	..	..	..	1	4	..	..	..	..	8	1	..	..	..	..	2	5	1	..	..	..	61	26
+ *B* + / *gl B sk*	self	..	1	2	14	8	4	..	..	..	..	2	2	..	..	..	4	0	2	..	..	..	1	4	1	45	25
+ *B′* + / *gl B sk*	× *gl B sk*	4	21	1	..	..	..	0	9	2	..	..	..	2	6	..	..	..	..	3	17	1	..	..	..	66	29
+ *B* + / *gl B sk*	× *gl B sk*	..	..	..	11	21	4	..	..	..	3	4	1	..	..	..	4	2	0	..	..	..	11	14	1	76	18

TABLE 2

Color-grade distributions and Gl_2-Sk *recombination in marked self progenies and crosses to* B *of* B′/B^b *and* B/B^b *comparator*

	Markers	+ +					+ *sk*					*gl* +					*gl sk*						
	Grade	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	Sum	Rec. pct.
+ *B′* + / *gl* B^b *sk*	self	23	5	..	..	..	4	1	..	..	..	5	1	..	..	.	8	1	..	..	..	48	24
+ *B* + / *gl* B^b *sk*	self	..	1	10	10	2	5	1	0	1	1	1	0	1	2	0	8	..	..	..	..	43	28
+ *B′* + / *gl* B^b *sk*	× *gl B sk*	6	7	1	..	..	..	..	1	1	1	..	1	2	..	..	..	..	..	9	6	35	17
+ *B* + / *gl* B^b *sk*	× *gl B sk*	..	..	..	3	1	..	..	..	2	..	..	..	..	3	..	..	..	.	1	.	10	..

and crossed to *gl B sk*, just as in the previously described test. In the selfs, B^b segregates were mostly grade 0; *B* individuals were higher in grade (Table 2). The classes of markers in the selfed *B′* test that distinguish *B′* and B^b did not differ in grade level; in the $B' \times B$ progenies, the class of markers (*gl sk*) bearing B^b was high in grade whereas the *B′*-bearing class (+ +) was low. B^b therefore segregates normally and is not affected by *B′*.

Tractability of a null mutable allele (B^v): In the B^v stocks only the glossy marker was immediately available for use, and segregation for *Pl-pl* (purple *vs.* sun-red) necessitated very careful analysis to insure that no confusion arose due to the new background. As before, paired hybrids of $+ B'$ and $+ B$ with *gl* B^v were selfed and crossed to *gl B*. The results were especially striking among *Pl* plants, which were graded on a scale of 0 to 7 for increasing color in this background. These *Pl* individuals are recorded in Table 3 (grades for B^v individuals indicate the intensity of the colored sectors). *B′* and $B^{v\prime}$ ("converted" B^v) plants were strikingly lower in color grade in selfs than their comparators. From the crosses $\times B$ it is clear that $B^{v\prime}$ is as strong as *B′* in its effect on newly introduced *B*; the converter capacity of $B^{v\prime}$ has been confirmed by subsequent progeny tests.

Phenotypes of maternal haploids in crosses involving B′: Haploid individuals arise in maize at frequencies ranging from less than one per 1000 to three percent or higher (COE 1959b). Such haploids are virtually all maternal in origin and arise from kernels in which the polar nuclei have been fertilized (COE, unpublished). The *B′* stocks by chance have incorporated lines having a history of high haploid frequency, and numerous haploids have been observed in these stocks. More than 20 haploids from crosses involving *B′* as maternal parent were observed during the past summer. Like their diploid sibs, all were weak in color. In contrast, four haploids arising from *B* maternal parent $\times B'$ have been observed and all were as intense as the *B* parent itself. Three were in a single progeny of 51 individuals and were graded 5, 5, and 6 among diploid sibs of grades 0 to 2. The remaining one was in a series of progenies (227 plants) having a less extensive history of high haploid frequency and was grade 6 among sibs of 0 to 2. Although these individuals had developed in intimate contact with a *B′*-carrying endosperm, they were unaffected by *B′*.

DISCUSSION

We may delimit a particulate hypothesis as follows: Plants called *B′* are genically *B* and carry an extragenic particle, **P**, which is maintained by *B* and is expressed in the suppression of pigmentation. In *b* gametes **P** is not maintained and disintegrates. These specifications will provide all the flexibility necessary to explain the basic observations and are no more difficult to envisage than are mechanisms of enforced copying of *B′* or of "transductor" units (see COE 1959a). None of the present data are inalterably incompatible with any of these hypotheses; however, the weight of evidence is against a particle.

Consider first the observation that haploids from $B \times B'$ do not receive the *B′* properties from the pollen parent even though all their diploid sibs do. Although

TABLE 3

Color-grade distributions and Gl_2-B *recombination in marked self progenies and crosses to* B *of* B′/B^v *and* B/B^v *comparator*

	Markers	+ *B*								+ *B*^{v}								*gl B*								*gl B*^{v}								Sum	Rec. pct.
	Grade	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7		
$\frac{+\ B'}{gl\ B^v}$	self	16	35	4	.	.	.	.	.	2	1	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	8	3	.	.	.	.	.	72	10
$\frac{+\ B}{gl\ B^v}$	self	.	.	.	.	.	3	6	12	.	.	.	.	.	.	2	0	.	.	.	.	.	.	.	2	.	.	.	.	.	.	4	1	30	15
$\frac{+\ B'}{gl\ B^v} \times gl\ B$		.	.	2	11	7	.	.	.	.	.	.	.	.	.	.	.	.	1	4	12	4	.	.	.	.	.	.	.	.	.	.	.	41	.
$\frac{+\ B}{gl\ B^v} \times gl\ B$		.	.	.	.	.	1	1	12	.	.	.	.	.	.	.	.	.	.	.	.	.	1	6	2	.	.	.	.	.	.	.	.	23	.

the possible anomalies in ovule and embryo sac development and in fertilization which might give rise to a haploid have not been categorized, in haploid formation normally a pollen tube certainly enters the embryo sac and discharges its sperms and cytoplasm; one sperm nucleus unites with the polar nuclei to form the triploid primary endosperm nucleus but probably the other sperm, if present, fails to unite with the egg cell. The egg cell would nonetheless be stimulated by the single fertilization to develop as a haploid. These assumptions are simple, logical modifications of the normal process of fertilization as summarized by KIESSELBACH (1949) and satisfy the observations of the maternal origin of haploids and of the occurrence of haploid embryos only in kernels with fertilized endosperms. On the basis of these considerations, in the cross $B \times B'$ a B egg cell that develops into a haploid was exposed at and after fertilization to intimate contact (possibly to actual cytoplasmic intermingling—see KIESSELBACH 1949, Fig. 54) with a B' B B cell. It is difficult to imagine that the postulated **P** would not be incorporated into ("infect") the cytoplasm of the embryo under these circumstances. If the picture of incomplete fertilization drawn here is correct, an extranuclear particle is unlikely to be involved in the B' phenomenon.

The demonstrations of an intractable but functionally intermediate allele, B^b, and of a tractable but functionally null (although mutable) allele, B^v, are also significant. If B maintains **P** (as required by the hypothesis), the means by which B accomplishes this should, for simplicity, be the means by which B functions; i.e., the product of B that maintains **P** should be the same as the product of B that elicits pigment formation. Otherwise, B would have an unexpected double function, one aspect of which is unexpressed except in the presence of **P**. But B^b, which has a degree of B-like function, does not maintain **P** in gametes (B^b is unaffected by B'), whereas B^v, although nonfunctional, can maintain **P** (B^v is changed to a converter by B'). These two alleles thus show properties contrary to the most simple considerations regarding a gene-maintained extragenic particle.

These observations do not exclude either a particulate or a genic system or a combination system as the basis for the B' phenomenon but are more strongly in favor of a genic system. Stocks in preparation should provide a definitive test.

SUMMARY

Extension of studies of the regular conversion-type phenomenon at the B locus in maize to marked heterozygotes, to two additional alleles, B^b and B^v, and to observations of maternal haploids among crosses of $B \times B'$ is reported.

Markers on either side of the B locus recombine, segregate, and are transmitted normally in crosses in which conversion occurs.

The intermediate allele B^b, like b, is intractable to B', whereas the functionally null (but mutable) allele B^v is changed, just as is B, to a "converter."

Haploids from the cross of $B \times B'$ are B in phenotype even though they have developed in contact with fertilized (genotypically B') endosperm.

These observations have been applied to a postulated system of a gene-main-

tained particle with the conclusion that this system is a less likely mechanism for the B' phenomenon than is a mechanism involving the gene more intimately. This conclusion is based on two considerations. First, in the formation of a haploid embryo in the cross $B \times B'$ the egg cell is likely to be exposed to the (particle-carrying) cytoplasm of the pollen tube, but maternal haploids from this cross have not received the hypothetical particle. Second, it is argued that the function of B that would maintain the particle should be the function that would elicit pigment formation, but a functionally intermediate allele (B^b) does not maintain the particle whereas a functionally null allele (B^v) does maintain it.

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ARE INDUCED MUTATIONS IN DROSOPHILA OVERDOMINANT? I. EXPERIMENTAL DESIGN[1]

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Background of the problem

IT seems likely (as pointed out by TYLOR in 1865, cited by DARWIN 1868) that primitive peoples had observed the tendency to lesser fitness manifested by the children of more closely related parents or, stated conversely, the tendency to greater fitness of the more crossbred, and that these observations were a factor in the widespread establishment of rules of exogamy. Lacking the genetic concepts for interpreting this matter, DARWIN (1859) thought of it as an expression of a principle, earlier proposed by HERBERT SPENCER, that living organizations tend to undergo stagnation (or to approach "equilibrium," as he put it) if continually subjected to unchanging conditions. Thus, the rejuvenescence that seemed to follow fertilization was thought to be more intense when the uniting germ cells were more (yet not too extremely) unlike, as stated, for example, by JOST (1904, 1907). Similarly G. H. SHULL regarded heterozygosity as a sort of stimulus when he originated his famous proposal for getting improved corn yields by taking the F_1 between selected inbred strains (1909, 1911) and EAST and HAYES (1911) held the same opinion. It is this ancient view of heterosis that is rightly to be termed the "classical" one, contrary to the practice of those (DOBZHANSKY 1955, WALLACE and DOBZHANSKY 1959) who have recently offered a version of it in more modern terms, that we shall refer to here as the "neoclassical" one.

However, even in the first two decades of this century the idea had already become prevalent among geneticists (see DAVENPORT 1908, and more especially BRUCE 1910; KEEBLE and PELLEW 1910; and ELDERTON 1911) that inbreeding leads to degeneration by bringing about the homozygosity of recessive, relatively deleterious genes, and that crossing acts conversely. (Neither BRUCE nor KEEBLE and PELLEW, however, indicated that they considered the dominant alleles in their scheme to be merely normals and the recessives as mutants, but ELDERTON seems to have taken this point for granted, and she even anticipated FISHER's hypothesis of the evolution of dominance.) On this view vigor was increasingly

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regarded as subject to the principles of multifactorial inheritance that were already being recognized as pertaining to quantitative characters (YULE 1906; LOCK 1906; MULLER 1914; MORGAN, STURTEVANT, MULLER and BRIDGES 1915). As COLLINS (1921) later showed, where numerous separately segregating loci are involved the production of an all-normal genotype is not to be expected, even apart from considerations of linkage, and the phenotypic frequency distribution will tend to be symmetrical, just as had been found for vigor.

EAST and JONES in their "*Inbreeding and Outbreeding*" (1919) gave an extended exposition of heterosis and related matters along these lines. They included, in addition, the important point made by JONES (1917) that linkage further tends to maintain heterozygosity by impeding the formation of recombinants that have only dominant normal genes (though unlike COLLINS they represented linkage as essential for the effects). The most extreme examples of this situation were those provided by balanced lethals (MULLER 1917, 1918). In connection with the latter it had, moreover, been pointed out (MULLER 1917, 1918, 1921) that mutations are in fact in the great majority of cases both recessive and detrimental, even degenerative, and that they are probably for the most part of minute, quantitative expression. WRIGHT also (1921) expressed essentially this view of the inbreeding-outbreeding contrast.

It happened, however, that EAST himself later (1936) proposed as a supplement to the above "neo-Mendelian" interpretation of the matter (as we shall here term it) the proposal that heterozygosity in alleles at an individual locus could, as by complementary action of the alleles, give rise to a fitness higher than that of either homozygote. Some time afterwards JONES (1944), followed by others, presented cases that he believed were of this type. HULL (1945, 1946), terming the postulated phenomenon "overdominance," and urging that it was of major importance in heterosis, then cited a series of results in crop plants that he believed presented evidence of it. He proposed that it might involve more than mere complementarity and thus brought the view closer to the ancient one of stimulation through difference *per se*. Some of these examples, however, involved pairs of alleles both of which were clearly mutants for different defects. As for the rest, it was seldom if ever possible to meet the rigorous genetic criteria required for proving that the individuals homozygous for either one of the alleles in question were not at the same time homozygous for detrimental recessives at other loci, especially at loci linked to that under consideration. In fact, this situation was the very one to be expected on the neo-Mendelian view. Reviews of the alternative hypotheses have been given by CROW (1948, 1952).

For somewhat over a decade there has been a reversion to the overdominance postulate among some geneticists. LERNER in his book *Genetic Homeostasis* (1954) suggested that "fitness as a whole exhibits the phenomenon of overdominance." Although leaving open the possibility that there are also "polygenic blocks" patterned after JONES's model, he states (p. 67) that "developmental homeostasis" (by which he means the stability of equilibrium in developmental reactions) is based on the "buffering of development by nonspecific action of

heterozygous gene pairs," though these nonspecifically interacting genes are assumed to be in specific and different groups for different characters considered. This seems to be a return, in modern terminology, to the old idea that difference is *per se* stimulating or somehow conducive to viability, or as EAST and JONES in 1919 put the matter (p. 169) when describing this idea, that there is "a physiological stimulation arising from the interaction of *different* hereditary factors."

WALLACE and DOBZHANSKY go even further and believe that the high proportion of deleterious alleles found to exist in populations "is most reasonably explained by the selective superiority of individuals heterozygous for two different forms of such alleles" (WALLACE 1958). As one would expect with such a view these authors question the legitimacy of calling an allele normal in the sense of fitness even when the allele under consideration occurs at so high a frequency as to have been termed normal in the sense of abundance. They consider that the viability of such an allele, when in a given environmental and genetic setting, would usually be less when homozygous than when heterozygous.

There is no doubt that such a situation exists for occasional loci, such as those of some blood antigens; but there is ground for remaining skeptical of its applicability to many loci, or to most alleles arising at any given locus. The neo-Mendelian interpretation, well based in theories of gene functioning, has been supported by significant observations made long after it had been put forward. Among these are the findings of the hypomorphic nature of most mutant genes, their incomplete recessiveness, and the peculiarities of dosage compensation (MULLER 1932). It has been argued (MULLER 1950; CROW 1952) that in most cases a situation of higher fitness of the heterozygote of a given locus would tend eventually to be superseded, through natural selection, by one of homozygosity in which the given allele had been suitably mitigated in its expression, as by modifying genes or intralocus reorganization, or had even been replaced by a condition of duplication having both alleles homozygous at once (*ibid.*, and HALDANE 1937). Calculations of the load on the human population (the loss of fitness as compared with the optimum combination of existing genes), that would be caused by the existence of many overdominant loci, moreover, have given results inconsistent with the findings regarding the loss of fitness associated with inbreeding (MORTON, CROW and MULLER 1956; CROW 1958). Furthermore, studies by MORTON and CHUNG (1959) have shown that for some specific cases investigated by them one would have to assume an unreasonably high number of overdominantly interacting alleles if one were to interpret their frequencies in the population by overdominance.

Despite these considerations, the present authors have felt that, in view of the results recently reported by WALLACE (1957, 1959) which he interpreted as confirming and extending the overdominance interpretation, a further experimental attack was advisable, using similar biological material. In a very large-scale experiment (involving some three and a quarter million flies) WALLACE found that a group of *Drosophila melanogaster* homozygous for a given unirradiated second chromosome had 1.5 percent lower average fitness than a second

group that did not differ in any consistent way from the first group except in having had one member of the given pair of second chromosomes previously irradiated with 500r in a spermatozoon stage, and in being to that extent more heterozygous. WALLACE (1958) concluded from these results that "more than half the alleles actually present in a given chromosome were seemingly improved by heterozygosity for a *randomly* induced allele" (italics ours). That is, the majority of the alleles induced at a given locus act with overdominance ("heterotically"). It is thereby suggested that most mutants are perhaps of only slightly or imperceptibly deviant homozygous expression, being in effect wild-type "isoalleles," and that relatively few give deleterious reactions when heterozygous (BURDICK and MUKAI 1958).

Since this view is mainly concerned with isoalleles it cannot be directly tested by the use of mutants having detectable effects. Few mutants that could be discovered by present techniques would have been produced by irradiation of spermatozoa with 500r. Even if we included as detectable those having, when homozygous, viability effects of as little as five percent but no visible effect we should expect (extrapolating from results of MULLER and MEYER 1959) that not more than one irradiated second chromosome in six would have one of these "detectable" mutant genes. If they alone had been the cause of the 1.5 percent increase in viability the average heterotic effect of any one of them would therefore have been of the order of nine percent. Such an effect, it might be thought, would be observable even if the "genetic background" of the treated chromosome instead of having been homozygous had been heterogeneous and heterozygous but comparable (i.e. not consistently different) in the two groups contrasted.

As a matter of fact, WALLACE did find, in his experiment, that there was a viability difference between two such groups having a heterozygous genetic background, and that it was of the same sign as and of a magnitude not much smaller than that found between his two otherwise comparable groups which had a homozygous genetic background in regard to their second chromosome. Since this result was not expected on his view, his findings seem to be at variance with both his own concept and the neo-Mendelian one. This consideration provided us with an additional reason for conducting a further experimental investigation of these matters.

Basic plan of present experiments

In the setting up of the plan and the construction of the stocks for the present series of experiments, by MULLER in 1956–58, it was decided to have the work of testing avoid crosses (like those of most previous studies) in which the chromosome under investigation had to be passed in many replicas through females heterozygous for a homologous chromosome containing inversions and markers, since occasional undetected crossovers and misclassifications happening in the course of such crosses could cause significant distortions in the final data. In each of the three major experiments of the present series, all of which were conducted on *Drosophila melanogaster*, the chromosome investigated was the third. A given

wild-type or nearly wild-type third chromosome, the "prototype," was first allowed to cross over, through one or two generations, with a third chromosome provided with suitably spaced markers, and a single final recombinant, to be called the "marked chromosome," was chosen that throughout was derived from the prototype except for a small region, not more than some five units long, containing a given recessive marker not present in the prototype. The marked chromosome and a single prototype chromosome, both derived from the same grandmother (in which and/or in whose daughter the given crossovers had occurred), were then subjected to crosses with stocks having inversions otherwise marked. Two stocks were thereby obtained, as rapidly as possible, one homozygous throughout for the marked chromosome and the other continually selected so as to be heterozygous in the males only for the marked and for the prototype chromosomes while in the females it was homozygous for the marked chromosome. In a part of the experiments the other major chromosomes (X and/or II) were also caused to be homozygous and alike in these two stocks, while in other cases, as will be explained later, they were replaced in the course of the testing so as to remove any consistent genetic differences that might be caused by these chromosomes between the two groups of flies that were to be compared.

After the establishment of the two stocks having their third chromosomes coisogenic except for the specially marked region the procedure was comparatively simple. Some flies containing the prototype chromosomes were irradiated and a comparable number of others were held as controls. A series of lines of descent, some "experimental," some "control," were thereupon established by crossing between these flies and flies homozygous for the marked unirradiated third chromosome. In any given line all the males chosen for breeding were always heterozygous for a given prototype third chromosome ultimately derived from a single irradiated germ cell or from a parallel unirradiated germ cell of a control fly of the same generation, as well as for a marked third chromosome derived from their mother. Moreover, the prototype chromosomes of different lines were always derived from irradiated or control germ cells that at the time of irradiation had been contained in different individuals. The males of each such line were in each generation backcrossed to females homozygous for the unirradiated marked chromosomes and counts were made of the numbers of unmarked and marked offspring in each culture. Among these offspring the unmarked males were again heterozygous and genetically like their fathers. Thus the operation could be repeated *ad lib.* if it was desired to increase the count for any or all lines. Comparison between the ratios shown by the experimental and treated lines was the final step for ascertaining what detectable effect on viability, if any, the induced mutations had produced.

It will be seen that in this experiment it is possible to have a much more direct comparison between the viability effects of irradiated and unirradiated chromosomes than in previously reported work, since this comparison can be made between flies (the marked *versus* unmarked ones) of the very same culture. This possibility arises from the fact that the two chromosomes here in question ordi-

narily differ, except in their small marked region, only in regard to such heterozygous mutant genes as may have been induced by the radiation, and that the cross is so constituted as to have flies with these two chromosomes both manifest their effects in the same culture. It is true that the effect of the difference in the small marked region must here be corrected for, by use of the data from the control cultures. On the other hand, the control cultures also supply information regarding effects on the ratios caused by differences in cultural conditions and in uncontrolled genetic influences (such as those due to spontaneous mutations and to possible pre-existing differences in the fourth and Y chromosomes). However, in the present work these other influences tend to be exerted alike on the flies of both irradiated and unirradiated classes of the same culture, whereas in previous works the corresponding classes were always in different cultures. Thus if there was any effect of a heterozygous mutant gene on viability it could have become evident in earlier work only by a comparison between the ratio manifested by it in its own culture or cultures and the corresponding ratio manifested by a control gene in a different culture or cultures. An additional way in which the present more simplified genetic technique enhances the determination of relative viabilities lies in the fact that in each test culture *all* the zygotes produced, instead of merely one quarter of them, as in most previous work, represent the genotypes of primary significance.

In order further to increase the significance of results involving any given total number of flies, it was decided to raise the effective dose as much as practicable, but in such a way as to leave the distribution of mutant types like that ordinarily obtained with comparatively low doses. In preliminary trials carried out separately by HELEN U. MEYER and by EHRLICH, under the direction of MULLER (MEYER, EHRLICH and MULLER 1959), it was found that flies of some vigorous types, of both sexes, could still give a few offspring when crossed to nonirradiated flies even after having received as much as 24,000r, provided that this was delivered in installments of 4,000r spaced at intervals of four days (the flies being kept at 26°C during the intervals). By taking offspring derived from spermatozoa or eggs released at least 12 days (usually at least 15 days) after the last irradiation it was insured that the germ cells used had been at a gonial stage during all irradiations. At this stage only about a sixth as many lethals are induced by a given dose as at the spermatozoon stage usually employed for irradiation, so that the 24,000r in this case give rise to about the same results, quantitatively, as are ordinarily obtained after a dose of 4000r to spermatozoa, and each installment of 4000r to the gonia gives results for lethals about like those of 700r applied to spermatozoa. However, far fewer minute rearrangements and incomparably fewer gross rearrangements of chromosome parts, in comparison with lethal and other point mutations, are induced by 4000r given to gonia than by 700r to spermatozoa.

Moreover, the relative frequency of the rearrangements is not raised by the increase in total dose, in our experiments, as it is in treatments of spermatozoa, for the broken ends of chromosomes produced by one installment of radiation

have undergone fusion in the four days intervening before the next installment is delivered. Thus the mutations induced by our irradiations must have consisted in a significantly higher degree of gene mutations than those in WALLACE's experiments did, despite the fact that the dose we used was some eightfold his dose in its point-mutation-inducing effectiveness.

If, now, we assume that both in the case of mutations that act beneficially when heterozygous and of those that act detrimentally their frequency rises linearly with total dose, and that the detrimental ones are at this level of frequency not significantly more additive or synergistic in their effects than the beneficial ones are, we must conclude that an increase in dose will not change the sign of the over-all effect but only its amount, rendering it more conspicuous. We believe, therefore, that our results have a level of significance about the same as would have been obtained in otherwise similar experiments involving at least eight times the total number of counted flies recorded by us, provided that those other experiments had been based on flies derived from spermatozoa irradiated with 500r.

Another technique designed to enhance the significance of our results was the use, in a part of the work, of cultures crowded in a standardized way. It had been found independently both by KERKIS (1935, 1938), working with MULLER (1934), and by TIMOFÉEF-RESSOVSKY (1934, 1935), and confirmed by later work, that small genetic influences on viability are expressed to a more marked degree when the competition is increased by crowding. Moreover, in some of our work relatively early counts of offspring were made. For, as BONNIER and JONSSON (1957) have shown, there is a strong positive correlation between viability and developmental speed, and it is also evident that in populations in which much competition occurs (as is often the case in Drosophila) speed of development would itself constitute an important factor in fitness. Finally, in some of our work a given line was subjected to several simultaneous tests, in order to make the statistical analysis more efficient and to throw light on whether the developmental stability had been affected in the lines with treated as compared with untreated chromosomes.

Three separate series of experiments following the general procedures above outlined have been carried out by the Drosophila group at Indiana University during the past three years. A preliminary experiment was carried out in the summer of 1958 under the direction of MULLER and with the assistance of MISS ELLYN COSDEN (now MRS. KORN), together with some supervision by DR. I. I. OSTER. A second experiment, on a much larger scale, was gradually prepared for by MULLER in 1958–59 and has been carried through under his direction by MRS. ELIZABETH EHRLICH, assisted recently by MISS CAROL BART. But although started earlier, it was not finished so soon, either on the experimental or mathematical side, as the third experiment. This large-scale experiment, which was performed by FALK with the assistance first of BART and then of EHRLICH, has already had some of its earlier results reported on in abstract form (FALK 1959). Because the calculations connected with it have already been completed while those of the

other two pieces of work are still being processed it will be reported upon next, and the other two experiments will be taken up in the third paper of our series.

SUMMARY

The background of the problem of overdominance and the plan of experiments to test for overdominance of induced mutations have been presented. The experimental work is in part presented in the following paper by FALK.

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ARE INDUCED MUTATIONS IN DROSOPHILA OVERDOMINANT?

II. EXPERIMENTAL RESULTS[1]

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IN the first paper of this series (MULLER and FALK 1961) the background of the problem of overdominance has been explained as well as the general organization of our experiments, designed to test to what extent induced mutations are overdominant. This paper will deal *in extenso* with one series of the experiments carried out and will discuss the question whether many overdominant mutants may be induced and maintained in a population. It will be shown that in spite of the very stringent design of the experiment, which gave every chance for "overdominant" mutants to show up, no evidence for their production at noticeable frequency was found. In fact the opposite proved to be true: although the differences found were too small to be statistically significant in consideration of the variance expected under the circumstances of the experiment, the results turned out to be in good agreement with those expected on the "neo-Mendelian" hypothesis, in that they indicated the average viability to be reduced in the flies heterozygous for induced mutations.

MATERIAL AND METHODS

For the present experiment pairs of third chromosomes of *Drosophila melanogaster* were prepared that carried the marker *ve* (veinlet, 3–0.2) and that were coisogenic except for the alleles *st* (scarlet, 3–44.0) and st^+, and the immediate neighborhood of this locus. The gene *st* had been introduced into only one of these two chromosomes by obtaining crossing over nearby *st* on each side of it successively: between *th* (thread, 3–43.2) and *st*, on its left, and between *st* and *cp* (clipped, 3–45.3), on its right. These pairs of chromosomes were the "prototype" and "marker" respectively as described in the previous paper (MULLER and FALK 1961). Males carrying these respective chromosomes balanced over a *ru h D InsCXF ca* chromosome were mated to their sisters of similar constitution and their progeny scored to determine the viability of the homozygotes. One pair of these coisogenic chromosomes which proved to be of good viability as homozygotes was chosen and males containing them were then crossed repeatedly to a

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large number of females from a stock homozygous for *cn bw*; *ri e*. All other pairs of chromosomes were discarded.

The scheme of the crosses is described graphically in Figure 1.

Several of the F_1 *cn bw*/+; *ri e*/ *ve st* males were mated singly to females having dominant markers and crossing over inhibiting inversions in their three large chromosomes, such as e.g. *y* sc^{81} *B In49* sn^{z2} *v* sc^{8} /+; *dp* ^{tz1} *CyIns05 pr* cn^{2} *T23 Me Ins ri* Sb^{1}/+ (the X-chromosome balancer having been prepared by MULLER and the autosomal translocation and the modification of the "Curly" inversions in the second chromosome by OSTER). By inbreeding the properly marked progeny of these crosses it was hoped that a viable stock isogenic for all genes in the normal X, normal second and *ve st* third chromosomes of an F_1 male could be obtained in F_4. During this procedure, however, it proved advisable to abandon the effort to isogenize the X chromosome because of the low viability of the F_2 males loaded with all the markers necessary for this. There resulted a contrast between the females and the males that were finally scored, in that the former were heterozygous, the latter only hemizygous with regard to invisible alleles in the normal-appearing X chromosome. The final stock, obtained in F_3, homozygous for its unmarked second chromosome and its *ve st* marked third chromosome, will be called the *ve st* stock. The stock was kept and multiplied separately during the rest of the experiment.
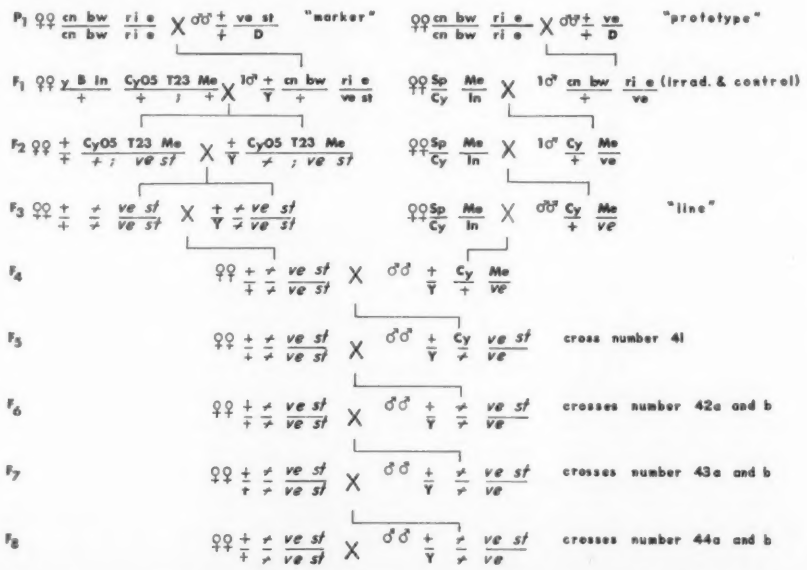
FIGURE 1.—Scheme of crosses. Abbreviations used: Cy05 T23 Me=*dp*^{tz1} *CyIn05 pr* cn^{2} *T23 Me Ins ri* Sb^{1}; D=*ru h D InsCXF ca*; In=*sep In ri* p^{p} *Sb*; Me=*Me InL InC e* l_3e; Sp=*dp*^{tz} *Sp cn*; y B In=*y* sc^{81} *B In49* sn^{z2} *v* sc^{8}. Isogenic chromosomes are in italics.

Simultaneously with the preparation of this *ve st* homozygous stock a great number of lines carrying individual irradiated *ve* chromosomes, derived from the *ve* chromosome that had originally been coisogenic with the given *ve st* chromosome, were obtained in the following manner. Nearly a thousand *cn bw* / + ; *ri e* / *ve* males were collected and were then divided at random into one group of over 500 and another of less than 500. The larger group was exposed to six doses of 4,000r, with an interval of five days at 18°C between the individual exposures. After the flies had received a total dose of 24,000r they were aged at 26°C for another 15 days. The smaller group of males were kept in the same way except that they were not irradiated at all. Both groups of males were transferred to new vials every 4–6 days and a group of virgin females, approximately equal in number to the males, was added once a week during the irradiation period and each 3–4 days during the aging period after the irradiations. The females were used in order to stimulate spermatogenesis.

The four first irradiations were given with the X-ray machine used for the past 15 years in our laboratory, operated at 200 kvp, 20 ma, with a 1 mm Al screen, at a rate of 160r/minute. The last two irradiations were given with a newly obtained "Maximar-100" machine, operated at 100 kvp, 5 ma, with a 1 mm Al screen, a target distance of 15 cm, and a rate of 220r/min.

At the end of the aging period when it could be inferred that sperm then being ejaculated had been irradiated in a spermatogonial stage, the males of both groups, which we shall design as F_1, were, as shown on the right-hand side of Figure 1, mated to females of stocks, previously constructed for such purposes by MULLER, that were properly marked and balanced as follows in their second and third chromosomes: *dp^{tz} Sp cn / Cy, Ins cn^2 sp^2 ; Me, InL InC e l$_2$e / sep,In ri p^p Sb* (or the equivalent). From any given F_1 male only one F_2 male, heterozygous for the two marked balancer autosomes and for the unmarked second and *ve*-marked third chromosome, was backcrossed to the marked and balanced stock. Male progeny from each such cross established a "line," in which males like the father and females like the mother were selected (*en masse*) for breeding. In this manner it was arranged that each irradiated male would contribute not more than one third chromosome to the experiment, and the possibility was avoided of clusters of identical irradiated chromosomes biasing the results.

By the time the progeny of the backcross F_3 were ready the *ve st* stock F_3 on the left-hand side of Figure 1 had been synthesized and multiplied. Virgin females from the *ve st* stock were now mated to *Cy cn^2 sp^2* / + ; *Me,InL InC e l$_2$e* / *ve* males of each irradiated and each nonirradiated line. Males derived from this F_4 cross which had the unmarked second chromosome from the *ve st* stock (here designated as $+_{180}$) were of composition *Cy cn^2 sp^2* / $+_{180}$; *ve st* / *ve*. These F_5 males were backcrossed to virgins of the *ve st* stock (cross number 41). Note that the unmarked second chromosome of these males was coisogenic with those of the *ve st* stock and that their pair of third chromosomes was homozygous and coisogenic with that of the *ve st* stock except for the *st* region and except for any mutations that had been induced in the *ve* chromosome (or that may have arisen

spontaneously since their common origin). In the case of each line, attempts were made to take for this cross (number 41 of F_5) 4–5 females from the *ve st* stock and about the same number of males from the previous backcross, but the number of parents could not be kept strictly constant. This was especially the case with lines which produced few offspring of the right type; every effort was made to establish such lines even if only one male could be used. The parents were allowed to remain in the vials for three days, then transferred twice successively to fresh vials for another two days each, after which they were discarded. All the crosses were kept in a 26°C incubator.

The progeny of cross number 41 were classified into four phenotypes of females and four phenotypes of males and counted separately for each line until the vials were exhausted. Males (F_6) of the phenotype veinlet derived from this cross and thus of genotype + / Y; $+_{iso}$ / $+_{iso}$; *ve* / *ve st* were kept separately for each line. Note that they were homozygous for the second chromosome (which was coisogenic with the second chromosome of the *ve st* stock) and that their X chromosomes were entirely derived from those of the *ve st* stock. Fifteen females of the *ve st* stock were then crossed to 15 phenotypically *ve* males of the above mentioned constitution from each line; where possible two crosses were made per line (crosses F_6 numbered 42a and b in Figure 1). In the case of many lines only one vial could be counted, either because not enough males of the right type for the cross had been secured from the previous generation, or because there was a suspicion of contamination in some of the vials to be counted.

Crosses 42a and b were kept for only one day in the 26°C incubator, after which the parents were transferred to fresh vials for two days for deposition of eggs, and the first-day cultures were discarded. It was assumed that, because the prior day had allowed mating, differences in numbers of eggs laid during this two day period were only negligibly due to differences in mating time. After the two days of egg laying the parents were discarded and the vials were transferred to an incubator kept at 18°C. The offspring were classified and counted twice: on the 27th day after beginning of egg laying and again on the 30th day. All progeny, except males of phenotype *ve*, were discarded. The males of the phenotype *ve* were backcrossed to females of the *ve st* stock, in a manner similar to that described above (crosses F_7 numbered 43a and b).

According to this plan it was intended to have counts of four vials for each line. In the majority of vials of crosses 42 and 43, 15 males could be provided, but there were a few in which only 14 or 13 males were available. It was found that this did not make any difference in the results so long as the full number of females was used. Furthermore, in a very small number of crosses even fewer males were available, some being done with only six or eight males. Here again, however, there seemed to be no indication for excluding them from the final calculations. It should be noted that not all lines were crossed simultaneously, and that two crosses of the same line were often prepared on different days.

In order to find out how far conditions at the 18°C temperature influenced the results one more cross (F_8 numbered 44a and b) was carried out with most of the

lines in the same manner as described above, except that the vials were kept in the 26°C incubator all the time and the flies counted on the 16th day after the beginning of egg laying.

It was obvious throughout the counts that there had been crowded conditions during development, especially since many flies were dwarfed and many others had a concave abdomen.

Throughout the experiment an effort was made not to select for the stronger lines. There is no doubt that in spite of this the lines lost did not represent a random sample. Out of 255 males which survived the irradiation and aging period, progeny were secured for the next step from 168; thus only two thirds of the males gave progeny. In the parallel group of controls only six out of 122 failed to give progeny, the success here being 95 percent. Since it was assumed that we would not be able to do counts on more than a hundred lines it was decided to keep some 70–80 lines from the irradiated group and a similar number from the controls, in the hope that at least 50 lines of each would be available for the final counts. Cross number 41 was started with 84 lines of the irradiated group and 71 lines in the nonirradiated group. Counts were in fact carried out on 73 and 65 lines, respectively, of these groups. Later there were only three losses of lines and a few cases of suspected contamination that caused six control lines to be lost. In general the procedure of the experiment was such that as soon as a vial was suspected of being contaminated it was discarded; this was necessary since in most cases there was no possibility of proving contamination by examination of the phenotypes present in the vial.

From the outset of the experiment, special consideration was given to the problem of accumulated spontaneous mutations. For this reason attempts were made to decrease as far as possible the number of generations between the isogenization of the chromosomes and the counts. The *ve* chromosomes were irradiated in the third generation after their preparation; the first count was done five generations after the irradiation and the three following counts in the three following generations. The *ve st* chromosome was re-established from a single heterozygous male three generations after its preparation. At the same time a single second chromosome for the stock was obtained. Five generations later the first count was done. The females for the cross number 42 were collected from the same generation as those for the cross number 41, and for the following generations they were obtained from successive generations of the *ve st* stock. Thus, not more than 12 generations elapsed between the time of separation of the *ve* and the *ve st* coisogenic chromosomes from one another and the end of the experiment.

In the case of most lines (but, because of technical difficulties, not all of them) the *ve* chromosomes had their viability determined not only in the heterozygous state but also in the homozygous one. For this purpose the chromosomes were transferred in F_5 or F_6 again to *Me / ve* males and females which were mated with one another and the proportion of *ve* among the total offspring of each of the lines determined.

CONSIDERATION OF RESULTS

The viability of the *ve* flies in each culture was estimated by comparing their frequency with that of the *ve st* flies of the same culture, assuming that the genotype of the *ve st* flies was identical in all cultures, and taking them as a reference group. For most of the calculations the proportion of *ve* females (or males) in a culture out of the total of the *ve* and *ve st* females (or males) in that culture was used as the measure of viability of the former. In cross number 41 there were in addition four phenotypes with Curly-wings, their viability was calculated separately, but with the same non-Curly *ve st* reference group. In this manner the effect of induced mutations in heterozygous condition on the viability of flies otherwise either homozygous or heterozygous with respect to their background could be estimated from the viability of the *ve* and *Cy ve* classes respectively. The corresponding viability calculations for the *Cy ve st* phenotypes gave another estimate of viability variations which were independent of radiation effects. There are two possibilities for choosing the *ve st* reference groups: (a) taking the *ve st* females as a reference group for the *ve* females and the *ve st* males as a reference group for the *ve* males, or alternatively (b) taking the same group, viz. *ve st* males, as a reference group for both *ve* females and *ve* males of a given culture. Usually the first method was used but in some calculations both possibilities were utilized.

Another estimate of viability can be obtained by the ratio of the *ve* flies of a given sex (and in cross number 41, also of a given wing shape) to the corresponding *ve st* flies in a given culture. As shown by HALDANE (1956) this ratio is a biased estimate. Still, the ratio of this ratio in the treated group to the same in the untreated group ("ratio of ratios") is a good and practically unbiased estimate of the effects of irradiation on the treated group (GREENBERG and CROW 1960). It is approximately equal to the number of lethal-equivalents induced. Analytically considered, this "ratio of ratios" is equal to e^{-KR}, where R is the number of roentgen units and K the number of lethal-equivalents per r-unit.

Table 1 gives the results of the series of crosses numbered 41. The number of flies counted and the mean viability of each genotype over all lines is given. In this series eight different genotypes were classified. Four genotypes were heterozygous for the *ve* chromosome but had a varying degree of heterozygosity of background. The two *Cy ve st* classes, like the two *ve st* classes, were presumably identical in all lines, irradiated and unirradiated, at least insofar as their third chromosome was concerned. In this table all calculations were made with the (non-Curly) *ve st* phenotype as reference group. As explained earlier, this gives an estimate of the viability of the treated groups with the contribution of the background heterozygosity practically eliminated as a cause of difference from the reference group in two cases (*ve* females and males) or present in the other two cases (*Cy ve* females and males). The number of flies counted in this cross was limited. Yet, the average viability of the flies heterozygous for irradiated chromosomes is lower than that of the controls though the difference is not sig-

TABLE 1

*Numbers of flies counted and the mean viability of lines with standard error for series 41**

Genotype	Degree of background heterozygosity	62† treated lines		61‡ untreated lines	
		Totals	Mean viability of lines ± standard error	Totals	Mean viability of lines ± standard error
$\frac{+\ +\ ve\ st}{Y\ +\ ve\ st}$	−	2070	0.500(arbitrary)	1919	0.500(arbitrary)
$\frac{+\ +\ ve\ st}{+\ +\ ve\ st}$	±	2326	0.500(arbitrary)	2138	0.500(arbitrary)
$\frac{+\ Cy\ ve\ st}{Y\ +\ ve\ st}$	+	2136	0.503±0.008	1886	0.500±0.011
$\frac{+\ Cy\ ve\ st}{+\ +\ ve\ st}$	++	2257	0.489±0.009	2044	0.495±0.008
$\frac{+\ +\ ve\ st}{Y\ +\ ve}$	−	2062	0.491±0.011	1821	0.493±0.018
$\frac{+\ +\ ve\ st}{+\ +\ ve}$	±	2413	0.511±0.008	2140	0.508±0.009
$\frac{+\ Cy\ ve\ st}{Y\ +\ ve}$	+	2175	0.508±0.009	2037	0.518±0.010
$\frac{+\ Cy\ ve\ st}{+\ +\ ve}$	++	2252	0.495±0.007	2110	0.501±0.011

* All viabilities are based on the proportion of the respective phenotype in each line in the total of this phenotype and the corresponding *ve st* phenotype.
† Eleven lines with less than 20 flies for at least one phenotype were excluded.
‡ Four lines with less than 20 flies for at least one phenotype were excluded.

nificant. It should, however, be noted that a number of lines which gave too few progeny (less than 20 flies in at least one of the eight phenotypic groups) were eliminated from the calculations. There is reason to believe that these included low viability lines, which, if included in the calculations, would increase further the difference between the treated and untreated groups. (A similar result is obtained when the *Cy ve* groups are compared with the *Cy ve st* as reference groups. This comparison eliminates the factor of the background heterozygosity and is parallel to the comparison of the *ve* groups with the *ve st* as reference groups.)

Three out of the four comparisons show a nonsignificant lower mean viability of the treated groups than that of the corresponding untreated groups and one shows a small increase in the average viability of the treated group. There is no indication of any correlation between the degree of background heterozygosity

and the differences in viability associated with irradiation. As a matter of fact, in the two groups with the higher background heterozygosity the differences between the means of the treated and untreated groups are greater than in any other comparisons. This together with the fact that the means of the less heterozygous groups had somewhat higher standard errors stresses the contribution of many minor deleterious mutants, which on being made homozygous caused both the lower initial untreated viability means and the higher variance observed in these groups (see also later). A more meaningful test for the radiation effects in this connection might be obtained by taking the difference between the mean viability of the *Cy ve st* phenotypes (as compared with the *ve st* phenotype, see Table 1) in the treated and untreated groups as a measure for the variations due to background and environmental factors. It can be seen that the average reduction of the mean viability of the four genotypes in the lower part of Table 1 in the treated groups, as compared with the untreated groups, amounts to 2.5 times the environmental and background variation.

Series 42 and 43 with their subseries were carried out in an identical manner. Series 44 was made in a similar manner to series 42 and 43 but raised at 26°C instead of 18°C as 42 and 43 had been. Calculations were done for the 27th day counts and the 30th day counts. The 30th day counts include all flies counted up to this day, i.e. including the 27th day counts.

Table 2 shows the results of the analysis of variance for the counts of the 30th day, taking into consideration all proportions available (a) for the whole material of series 42, 43 and 44, and also (b) separately for the untreated and treated lines. This analysis makes possible a comparison of the variance between lines with that calculated from the average variance among the cultures within the same line, on the assumption that there are only random differences between lines. In a similar manner it makes possible a comparison of the variance between lines of the treated and the untreated groups (and also between the average of the lines raised in one temperature and that of lines raised in the other temperature within the treated and untreated groups, respectively, as well as between the averages of lines for males and for females within each temperature and treatment group) with that calculated, on the assumption that there are only random differences between treated and untreated means, from the average variance found among the lines within each treatment (or temperatures or sexes, as the case may be). Thus, we were testing whether the variance between the averages of two compared groups was larger than that calculated from the variance within the groups.

Table 3 gives the number of flies counted in these series and the mean of the proportions of *ve* flies in the different lines. The proportion for each line was a weighted mean of its proportions in its subseries.

A glance at Tables 2 and 3 would indicate that for most comparisons the differences were not significantly larger than expected by chance from the variance found within the groups compared. Although this might seem disappointing it was not unexpected. It should be kept in mind that all flies were homozygous for most of their genes, thus many recessive viability-reducing genes, present in the

TABLE 2

Analysis of variance for viability calculations based on counts of the 30th day and with the ve st *females as reference class for* ve *females and* ve st *males as reference class for* ve *males*

a. Analysis of variance for the whole material of series 42, 43 and 44.

Source of variance	Degrees of freedom	Mean squares	F	P
Between treatments	1	0.002152	0.616	....
Between temperatures	2	0.067084	19.205	<0.005
Between sexes	4	0.001760	0.504	....
Between lines	486	0.003493	0.944	>0.05
Between repetitions	808	0.003701	....	....

b. Analysis of variance for the untreated and for the treated lines for the series 42, 43 and 44.

Source of variance	Degrees of freedom	Mean squares	F	P
Untreated				
Between temperatures	1	0.046905	15.038	<0.005
Between sexes	2	0.001736	0.557	....
Between lines	214	0.003119	1.102	....
Between repetitions	350	0.002829	....	....
Treated				
Between temperatures	1	0.087264	23.196	<0.005
Between sexes	2	0.001784	0.474	....
Between lines	272	0.003762	0.861	....
Between repetitions	458	0.004368	....	....

original chromosomes, expressed their effect. In other words, from the outset our viability values were in the rather subnormal part of the viability scale. Since subnormality is connected with developmental instability (MULLER 1950b; LEWONTIN 1957; SPASSKY, SPASSKY, PAVLOVSKY, KRIMBAS, KRIMBAS and DOBZHANSKY 1960) it is not surprising that great variance between repetitions of similar lines was found and that this practically overrode any variance due to other sources. We are therefore in a somewhat paradoxical situation. On the one hand we have to keep the environmental variance component low in order to be able to detect small differences in genetic components, but simultaneously, for the possibility that overdominant mutations with only small effects are induced, we are trying to keep the lines highly homozygous so as to have a better chance (according to advocates of the overdominance hypothesis) of detecting on this genetic background small effects of viability due to heterozygous induced mutations. Keeping this in mind the analysis seems to be rather unequivocal.

The variance between lines is about as high as that calculated from the variance within lines (i.e., between repetitions of the same lines) (Table 2). This is what one would indeed expect to find in the untreated group since, if isogenization has been effective, the difference between two lines should not be greater than that between two repetitions of the same line. On the other hand, if irradiation affected

TABLE 3

*Numbers of flies counted and mean viability of lines with standard error for series 42, 43 and 44**

	Temperature raised	Series and days of count	Number of lines tested	Females I		Females II		Males	
				Totals	Mean of lines ± standard error	Totals	Mean of lines ± standard error	Totals	Mean of lines ± standard error
Untreated	18°C	42 & 43 27th	58	14,476	0.526±0.007	13,751	0.551±0.006	13,163	0.531±0.005
		30th	58	16,592	0.517±0.007	15,808	0.537±0.007	15,223	0.521±0.005
	26°C	44 16th	51	8,846	0.498±0.007	8,371	0.530±0.008	7,875	0.497±0.005
Treated	18°C	42 & 43 27th	71	17,577	0.525±0.004	17,098	0.541±0.004	16,300	0.516±0.004
		30th	71	20,916	0.514±0.003	20,269	0.531±0.004	19,419	0.510±0.004
	26°C	44 16th	67	10,612	0.496±0.005	9,911	0.530±0.007	9,170	0.488±0.007

* Female viabilities taken with *ve st* females as reference group (I) and *ve st* males as reference group (II).

TABLE 4

*Radiation effects in series 42, 43 and 44 expressed as "ratio of ratios" and lethal-equivalents**

Temperature	Series	Day of counting	Females I		Females II		Males	
			Ratio of ratios	Lethal-equivalent per $r \times 10^6$	Ratio of ratios	Lethal-equivalent per $r \times 10^6$	Ratio of ratios	Lethal-equivalent per $r \times 10^6$
18°C	42 & 43	27	1.018	—0.82	0.918	3.57	0.866	5.99
		30	1.021	—0.82	0.985	0.63	0.975	1.06
26°C	44	16	0.982	0.76	0.940	2.58	0.981	0.80

* Females taken with *ve st* females as reference group (I) and *ve st* males as reference group (II). "Ratio of ratios" = ratio between the ratio of the *ve* to *ve st* in the treated group and the corresponding ratio in the untreated group. Average effect: 3.5 percent reduction in viability or 1.5×10^{-6} lethal-equivalents per roentgen unit.

the viability of heterozygotes for irradiated chromosomes, we might expect a change in the variance of the treated group. If heterozygosity for induced mutations increased viability it would have increased also developmental stability ("homeostasis"), so that one would expect a decrease in variance between repetitions in the treated group as compared with the untreated group. If irradiation caused mutations which affected the viability of different lines to different extents, it would increase the variance between lines. It seems clear that, if anything, the irradiation caused rather an *increase* of variance between lines and even more so within lines; thus indicating a detrimental rather than a beneficial effect on viability.

The absence of significant differences between the means of the sexes (Table 3) indicates that the smaller background heterozygosity of males had little influence on either the viability of the treated group or that of the control group. This is also borne out by the agreement of the estimate of variation between sexes with that calculated from the variance between lines within each sex (Table 2).

A highly significant difference was found between the series raised at 18°C and those raised at 26°C (Table 2). All means, of the treated as well as of the corresponding untreated groups, were *lower* in the higher temperature series (Table 3). The reason for this seems to be at least partly that there is a difference in speed of development between *ve* flies and *ve st* flies, the latter being slower. This is borne out by the data of Table 3; within the series raised at 18°C the means of the 27th-day counts were higher than those of the 30th-day. Thus, it seems that 16 days at 26°C correspond to more than 30 days at 18°C, unless temperature affects these classes unequally.

The variance of the differences between the averages of the treated and untreated groups is only of the same order of magnitude as that calculated from the variance between lines (Table 2). Thus the change in the viability caused by introducing one irradiated chromosome into the genotype was too small to be statistically significant in conjunction with the other components of variance. The same conclusion can be drawn from Table 3. The differences between the means are too small when compared with their standard deviations. Still, comparing any two corresponding values in the table, the mean viability is in all but one case lower in the treated group than in the untreated group. These differences in the comparisons made with females average about 0.5 percent decrease in the mean viability of the treated group and in the males reach about a three percent decrease in the 27th day count at 18°C (the percentage here given being that which the difference forms in relation to the value for the untreated group).

Furthermore, for the males, the earlier the counts were made the greater the difference between the treated and untreated group (it has been noted above that the 16th-day counts of the 26°C series probably correspond to counts later than the 30th-day counts of the 18°C series). Thus, for the earliest count, that of the 27th-day of the 18°C series, the difference between groups is on the verge of significance ($t = 2.29$, $P = 0.02$). The selective importance of speed of development

in Drosophila populations has already been pointed out (as by MULLER and FALK 1961).

Table 4 shows the effect of the irradiation on the heterozygotes expressed as lethal-equivalents. The average decrease of viability is 3.5 percent; that is, 1.5×10^{-6} lethal-equivalents per roentgen unit were induced. The order of magnitude of the lethal-equivalent per roentgen is about the same as that obtained by FRIEDMAN and CROW (1960) in their experiment; but since they applied the radiation to mature sperm our induced effects in spermatogonia would be expected to be lower.

The question might be raised whether the difference between the treated and untreated groups is not too small in comparison with the expected according to the "neo-Mendelian" hypothesis. Since recessive lethals have an average dominance of somewhere between three percent and five percent (STERN, CARSON, KINST, NOVITSKI, UPHOFF 1952; MULLER and CAMPBELL 1950) is it possible that these results reflect simply the net effect of heterozygotes for lethals in decreasing the mean viability taken in conjunction with net effect of heterozygotes for nonlethal mutations in increasing the mean viability? However, a little consideration shows that quite apart from the *a priori* improbability of a qualitative difference between lethals and nonlethal detrimental mutations, the data do not support such a possibility. As shown earlier, analysis of variance did not reveal so large an increase of variance between the lines in the treated groups, as compared with that in the untreated groups, as would be expected on such an explanation.

More evidence regarding this possibility is included in Table 5. Most of the *ve* chromosomes were tested for the presence of lethal mutations. Out of 61 treated chromosomes 20 carried recessive lethals, while only one out of 53 control chromosomes carried a recessive lethal. Thus irradiation induced recessive lethals in about 30 percent of the treated chromosomes. This is in good agreement with the expected frequency (MEYER, EHRLICH and MULLER 1959). Thirty-seven treated chromosomes and 42 untreated chromosomes were tested further to determine the viability of homozygotes, expressed as the percentage of *ve* flies in the cultures. The mean viability of the homozygotes for the treated chromosomes was only about 2/3 that of the untreated ones. Of this reduction over 90 percent was due to lethals and somewhat less than ten percent was due to nonlethal mutations induced by irradiation.

It should, however, be noted that if the effect of the mutants was dominant to some degree, which appears to have been the case, this is a minimum estimate of the proportional part played by nonlethals in the reduction of viability. That is because the viability estimate was based on the ratio of homozygotes for the relevant chromosome to heterozygotes for the same chromosome and for a tester chromosome. Any dominant effect would reduce the frequency of the heterozygote class to some extent in the same direction as the homozygote class, and thus would tend to cause an underestimation of the deleterious effect of the nonlethal mutations (see GOLDSCHMIDT and FALK 1959). The lower part of Table 5 gives the mean viability of the heterozygotes for those chromosomes the mean

TABLE 5

Radiation effect on homozygotes and heterozygotes

	Untreated	Treated
Test of Homozygotes		
Number of tested lines	53	61
Number of lethal lines	1	20
Percent of lethals	1.9	32.8
Mean Viability of Homozygotes		
All tested lines	0.333	0.207
Lethals excluded	0.342	0.333
Mean Viability of Heterozygotes		
All lines	0.518	0.512
Lethal lines	..	0.516
Nonlethal lines	..	0.510

Note: The scale of viability for homozygotes was different from that of heterozygotes—see text.

viabilities of which were also determined as homozygotes. (These are means of the estimates of lines, each line based on the weighted values of all its subseries done at 18°C and counted on the 30th day, females and males pooled.) It is clear from this table that the effect of 41 nonlethals in reducing the viability of the heterozygotes was as intense as that of 20 lethals. The viability of heterozygotes for lethal mutations seems, as a matter of fact, to be too high when compared with that found by STERN *et al.* (1952) and by MULLER and CAMPBELL (1950), but this might be due to the large inherent error. Actually, the difference between the mean viability of the treated and the untreated groups ranges from practically zero to about three percent and is well within the limits of the 3–5 percent reduction of viability expected of heterozygotes for recessive lethals. This result, although statistically not significant, is in line with the suggestion that the degree of dominance of nonlethal mutations is greater than that of lethals (MULLER 1950; GREENBERG and CROW 1960; JAMES 1960) and rules out the possibility of an effect on the mean of lines heterozygous for nonlethals acting in the opposite direction to lines heterozygous for lethals.

Since the results do not show a *significant* decrease in viability of the heterozygotes for induced mutations it is of some interest to calculate how much *increase* in their average viability would be consistent with these results. If we reduce by 1.4 times its standard deviation the mean of the untreated group where the smallest difference between treated and untreated groups was observed (series 44 females when *ve st* males are taken as the reference group) and also increase by 1.4 times its standard deviation the mean of the corresponding treated group, we get the maximum increase in viability compatible with our results. This amounts here to about four percent. WALLACE (1959) got an increased viability of 1.5 percent by irradiating the spermatozoa with 500r. The effect of that dose corresponds to about a seventh of the effect of the 24,000r dose given here to the spermatogonia (MULLER and FALK 1961). Thus, if the increase in viability is

proportional to the number of mutations induced, the maximum possible increase in our experiment would correspond to an increase of about 0.6 percent for WALLACE's experiment. From WALLACE's paper it is possible to calculate that the 1.5 percent increase in viability had a standard error of about 0.6 percent, and hence the lower limit of the effect he got is larger than the maximum increase at all possible in our experiment. Therefore, even when the most extremely favorable assumptions are made one cannot reconcile our results with those of WALLACE.

WALLACE (1958) lists a number of factors which could possibly have caused an increase in the frequency of a given class of flies so as to produce an effect simulating an increase in viability. He believes he excluded, among others, the possibilities that the effect is due either to misclassification or to gametic-ratio distortion. These points, however, deserve some re-examination. In the presence of an additional Y chromosome the marker Plum will be classified as wild type (BRIDGES and BREHME 1944), and genic modifiers can also normalize it to some extent (MULLER, unpubl.). Since some nondisjunction as well as gene mutation was probably induced by irradiation it is possible that there were more misclassifications of *Pm* as non-*Pm* in the irradiated series than in the controls. At least some of these misclassifications might not be eliminated even after another testcross.

The original flies for WALLACE's experiments were taken from a population cage which had an irradiated history. A small test was done to determine the possibility of a segregation-distorter being present. Recently NOVITSKI and HANKS (1959) found at least two Segregation-distorter genes in the flies originating from the population cages of WALLACE. If indeed there were segregation-distorter genes present and if they had such capricious behavior as the *SD* gene described by SANDLER and HIRAIZUMI (1959), then it is very possible that such a gene (or genes) was missed in WALLACE's search. Of the various series of experiments carried out by WALLACE only two (2–9M and 15–22F) showed a significant increase in viability of over two percent (WALLACE 1959). All other experiments had an increased viability of the +/+ group of the same order of magnitude as that of the *Cy*/+ or *Pm*/+ group, respectively. This heterogeneity might indeed be due to the occurrence of segregation-distorters in some cultures and not in others. Furthermore, in his earlier paper WALLACE (1958) reports an average increase of viability of 2.5 percent while in the later paper (1959) an increase of 1.5 percent was mentioned. This would suggest that the claimed increase in viability was found only in the earlier experiments while it was absent from the later series. One wonders therefore if in spite of the large scale of his experiments, a chance deviation was not responsible for the results.

DISCUSSION

It was the purpose of this paper to obtain some evidence regarding the mechanism underlying heterosis: in particular, whether it is due in any considerable degree to interallelic interactions giving overdominance or, on the other hand, to

simple dominance-recessivity relations between genes concerned with viability. The experiment presented here supports the notion that induced mutations are essentially deleterious, whether in the homozygous or the heterozygous state. Even under the most favorable conditions for their detection, mutations increasing the viability of the heterozygote, whether due to dominance or overdominance, were, to say the least, extremely rare.

It is unfortunate that with a few exceptions—notably WALLACE's papers (1958–1959)—much of the work taken to support the "neoclassical"-overdominance hypothesis of heterosis is not crucial since it is compatible with both theories when considered at the level of the gene. It may be of some interest to review shortly the possibilities for overdominant gene interactions and their chances of becoming established in the population, as well as to examine under what assumptions one can interpret the heterosis observed in natural populations as due to overdominant gene interactions and the load of the population as due to the segregation of the less adapted homozygotes (segregation load, CROW 1958).

Studies done by a great number of workers have made it clear that for many genes which seemed to be recessive, the heterozygote was somewhere intermediate between the homozygotes. In other words, they were dominant to some degree. This has been shown in many organisms, but mainly in Drosophila, especially for genes affecting viability (MULLER 1950b; STERN *et al.* 1952; MULLER and CAMPBELL 1950; FALK 1955 for newly induced mutations, and CORDIERO 1952; PROUT 1952; GOLDSCHMIDT and FALK 1959; HIRAIZUMI and CROW 1960 for mutants in natural populations), and also in yeast (JAMES 1960) and in man (LEVIT 1936; NEEL 1949). It may be noted also that Table 2 of DOBZHANSKY *et al.* (1960) suggests that nonlethal viability-reducing mutations on chromosomes free of lethals had on the average a higher relative degree of dominance than lethals (however, these authors regard the table as evidence for a more or less opposite argument, due to their taking the "average heterozygote" as the "normal" which serves as the reference point for their comparisons). On these grounds it is reasonable to assume that even those genes tested which did not show deviation from complete recessiveness were for the most part dominant, to a degree too small to be detected by present techniques. But it is also plausible that besides some fully recessive genes there are some which show overdominance (STERN *et al.* 1952; ALLISON 1955 for examples; and MUKAI and BURDICK 1959).

Many attempts have been made to classify the possible mechanisms for overdominance at the gene action level. Essentially they can be reduced to three types as suggested by CROW (1952) and ALLISON (1959).

1. One allele is responsible for the production of a certain material while the other allele does not produce any, or only a small amount of it. If only an optimal amount of material is required the heterozygote may produce an amount nearer to the optimal than either homozygote, as is the case in the sulfanamide-requiring Neurospora (EMERSON 1948) and perhaps also for cases like the absence of catalase from erythrocytes of dogs (ALLISON, APREES and BURN 1957).

2. The alleles each produce a different effect, both of which are important for

the individual, as in the case of sickling in man (ALLISON 1955) and in some cases of rust resistance in flax (FLOR 1947).

3. The heterozygote produces a primary product different from that produced by both homozygotes. This might be the case with some antigens, such as the AB antigen in man (MORGAN and WATKINS 1956) and the "hybrid antigen" in pigeons (IRWIN 1947) and rabbits (COHEN 1956).

It should be noted that the difference between types 2 and 3 may be mainly a theoretical one, because it is usually impossible to eliminate the possibility that the "hybrid product" is not the primary product of the alleles. SMITHIES and co-workers' study with haptoglobins is a case in point for, although they found a hybrid haptoglobin produced by heterozygotes (CONNELL and SMITHIES 1959), further analysis demonstrated this haptoglobin to be a secondary derivative of two products similar to those produced by each of the homozygotes. Thus the third type, if it exists, is the only one which comprises real interallelic interactions, while the first two confirm the rule that most genes are not completely recessive; in them, heterosis is present only on the phenotypic level from the point of view of fitness.

Since genes and their alleles are under the pressure of natural selection it is to be expected that "the exceptional cases of the heterozygote being superior are probably represented for the most part . . . by adaptations that have not yet stood the test of geologic time. For we should expect natural selection in the end to find mutations giving gene combinations which can achieve in homozygous form the same beneficial effects that at first could be attained only by the unstable, wasteful means provided by the selection of heterozygotes" (MULLER 1956).

Different authors (cf. MULLER and FALK 1961) have pointed out simple, known mechanisms which would turn the loci showing overdominant interactions into genes—or gene complexes—showing a regular dominance-recessivity relationship. The selective pressure for such a change is very considerable as has been shown by HIRAIZUMI and CROW (1960).

Thus, all evidence from the level of gene action makes it highly improbable that there exists on a large scale a system based on more or less nonspecific interactions of pairs of individually ill-adapted alleles giving adaptive combinations.

Turning to the populational level, an elementary notion must be borne in mind when dealing with overdominant allelic interactions: "The loss of fitness of the population (with a superior heterozygote) is of the order of magnitude of the selection coefficient, as HALDANE (1937) has shown, whereas with a detrimental recessive the loss is of the order of the mutation rate. Hence a single overdominant locus has a tremendously greater effect on the population fitness than a single locus with dominance and intermediate heterozygote" (CROW 1952). This in itself might raise the suspicion that a model based on a high proportion of loci having heterotic alleles will actually lead to conflict with observational evidence on the structure of the population, since the effect of many overdomi-

nant genes should consist in a much greater loss of fitness than any population might withstand.

MORTON, CROW and MULLER (1956) estimated the total mutational damage in human populations to be about 3–5 lethal-equivalents per zygote. They showed that it was impossible to attribute the load of the population to a "segregation load" from adaptive heterozygotes for a single pair of alleles per locus concerned. If it was assumed, however, that there exist many alleles at each locus, all of them (or at least a great proportion of them) interacting heterotically, the observed load could still be mainly a segregational one (CROW 1958). Studies by MORTON (1959, 1960) showed that for some malformations one has to assume the presence of some dozens to a few hundred alleles in each locus considered, all of which interact heterotically in virtually all combinations, in order to be able to attribute the observed load to "segregation load." Essentially similar results were obtained by GREENBERG and CROW (1960) when this type of calculation was extended to data available from Drosophila populations of different species. As shown earlier and as will be shown also below this huge number of alleles per locus interacting heterotically is a most improbable assumption.

A more direct demonstration of the absence of any considerable degree of overdominant interactions in determining heterosis was given by SIEGEL (1958) for *Paramecium aurelia*. In this species inbreeding predominates, although occasional advantage is taken of hybridization. Heterosis is demonstrable in the laboratory but exists also in natural populations. He demonstrated that some F_2 which were produced by autogamy from F_1 hybrids showed nearly as much vigor as the heterotic F_1's. Since these animal clones were completely homozygous, this result could be due only to recombinations of advantageous genes—as expected according to the "dominance" hypothesis and in sharp contrast to the expectation for the "overdominance" hypothesis.

Another difficulty makes a model based on many alleles at given loci interacting heterotically quite improbable. At each locus where there are two or more alleles present there is a probability of fixation of one of the alleles merely by chance. In an organism the adaptiveness of which is based to a great extent on keeping many alleles at each locus there would during each generation be a large loss of variability due to chance fluctuations, especially when it is noted that the frequency of each allele cannot be very high. This has been noted by WALLACE and DOBZHANSKY (1959). In that case, as they also note, mutation should have a constructive value in compensating for the chance losses of fitness caused by this elimination of alleles, and the induction of more mutations by radiation might in that case have an over-all directly beneficial effect. CROW (1960) pointed out that the theoretical question of the effect of mutations on fitness in a system like that in a finite population is a difficult one. In view of the evidence accumulated over so many years (to which DOBZHANSKY and his group contributed so much) as to the importance of selection in determining the fitness of populations, it is hard to believe that fitness is due in a great extent to a balance between random drift and random mutation.

It would seem to follow from the above results and considerations that the variability, polymorphism and heterosis of natural populations is not due to a large extent to many loci with overdominant alleles. As shown, this view is not only improbable on the basis of considerations based on gene function and because of the contradictions to which it leads when the structure of populations is analyzed critically but also in view of the results of direct experimental attack.

SUMMARY

The experiment described was intended to investigate whether induced mutations when in heterozygous condition and unselected can increase the average viability of an otherwise homozygous genotype. The design of the experiment (the general lines of which were given in the previous paper of this series by MULLER and FALK 1961) was such as to afford every chance for an induced increase in viability to be detected.

More than 60 lines were prepared, each having an irradiated third chromosome marked with the gene *ve*, carried along with a *ve st* marked chromosome (coisogenic—except for the *st* locus and its vicinity—with the *ve*-marked chromosome before its irradiation), and homozygous as well as isogenic with respect to the second chromosome genes. A similar number of control lines, identical to those irradiated in all details except for the irradiation, were also secured.

Males from all lines were repeatedly outcrossed to females from a stock homozygous for second and third chromosomes which were also coisogenic with the unmarked second and the *ve st* third chromosomes of the treated and control lines.

The irradiation delivered to the treated lines comprised six doses of 4000r each, given to males at five-day intervals. The irradiated males were kept for 15 days after the last irradiation before deriving from them the offspring studied, so that practically only sperm that had been irradiated at spermatogonial stages was utilized.

In each line the viability of the *ve* flies heterozygous for induced mutations, or the viability of the corresponding controls, was determined from the proportion of *ve* females or males out of the total of the respective *ve* and *ve st* phenotypes, the latter serving as a reference group for determining the viability of the former. In one backcross, where the male parents were heterozygous for the *Cy* marked chromosome and the isogenic second chromosome, viability was estimated for the *Cy ve* phenotypes separately but in a similar manner to that of the *ve* phenotype.

The lines were raised under standardized crowding conditions at 18°C or at 26°C on different occasions.

As could be expected from backcrosses of lines made homozygous for so many genes, including minor deleterious genes, the variability between repetitions within lines was rather high. This variability overrode most differences—which were expected to be rather small—between the treated and the untreated-control groups. Nevertheless, in nearly every possible comparison the treated lines had a lower mean viability than the untreated lines. The reduction of viability caused

by the presence in heterozygous condition of radiation-induced mutations ranged from zero to three percent.

The reduction in viability was more pronounced in earlier counts than in later ones of the same crosses, a result indicating that not only viability but also rate of development was adversely affected by the irradiation.

The reduction of viability found among heterozygotes for nonlethal-bearing chromosomes was about the same in extent as that expected for the lethal-bearing heterozygotes (the observed reduction of viability caused by the heterozygous lethals was smaller than that found for heterozygous nonlethals and than that expected for heterozygous lethals). In the homozygotes, on the other hand, 90 percent of the reduction of viability was due to lethals and only about ten percent to nonlethal detrimentals. This result may be taken as support for the notion that nonlethal deleterious mutants are on the average relatively more dominant than lethals.

The reported results were analyzed to find the maximum increase in viability statistically compatible with them. This was found to be even lower than the minimum increase possible according to WALLACE's (1959) experiments, in which he found an average increase of 1.5 percent in the viability of lines heterozygous for radiation-induced mutations.

The possibilities of inducing many overdominant mutations and maintaining them in a population are discussed.

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MUTABLE a_1 OF THE En SYSTEM IN MAIZE[1]*

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MUTABLE genes in maize are under the control of identifiable components that exhibit an unusual behavior. These components control the loci with which they are associated in that the action of a particular locus becomes altered when the component is intimately associated with it.

Characteristics of mutable genes include variable rates of mutability which are heritable and can be recognized as distinct pattern types, specificity between members of a mutable system, diverse pathways of mutation and the transposition of mutable gene components from one position to another in the genome. It is hypothesized that normal gene action is inhibited by controlling elements (*Ds*, McCLINTOCK 1951; *Mp*, BRINK and NILAN 1952; or *I*, PETERSON 1960a) when they are located in the vicinity of the affected locus. In certain cases the presence of a second element (such as *En*) is necessary before any expression of mutability can be discerned; i.e., in the absence of the second element, these inhibited loci appear to be stable recessives (Figure 2h).

The components of a mutable system exhibit a high degree of specificity. This is evident in the relationship between *Ac* and *Ds* in one system and *En* and *I* in another system. In addition to these, there are other two-element systems of mutability control such as a_1^{dt}–*Dt* (RHOADES 1941; NUFFER 1955) and a_1^{m-1}–*Spm* (McCLINTOCK 1956). The activators (*Ac*, *Dt*, *Spm*, *En*) do not affect any but their own specific inhibiting component (except in the case noted below) even though the same locus may be involved, such as the a_1 locus on chromosome 3. Each system is highly specific. For example, in tests of the interrelations of systems it was found that *En* does not cause the instability of McCLINTOCK's *Ds* component. There is, however, an interrelationship between *Mp* and *Ds; Mp* affects *Ds* instability (BARCLAY and BRINK 1954) and *Ac* affects P^{vv} mutability (BRINK 1958a).

Another characteristic of mutable gene systems is that the mutable components may move from one location to another, a feature which McCLINTOCK termed transposition. This is recognized in mutable gene stocks where stable standard dominant alleles at other loci have been observed to have become mutable (McCLINTOCK 1951, 1953). In such a case, the problem is to determine if these new mutables respond to the components of the specific system in whose presence

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they arose. If this proves to be the case, such evidence would strongly suggest transposition—the transfer of mutable gene components from a mutating locus to a stable one.

In this paper, a new mutable which arose in a pale green mutable stock (*I–En* system) is described. In addition, it will be demonstrated that it responds to the components of the Enhancer system. Abstracts of this material have been reported (PETERSON 1957, 1960b).

MATERIALS AND METHODS

The new mutable, a_1^m, arose in a pale green mutable stock. The mutability of a_1^m is recognized by the mutation of the a_1 allele to A_1 (colorless to colored—purple or red depending on the *pr* locus) and appears in both sporophyte and endosperm tissue. The a_1^m allele may also change to a nonmutating colorless allele. It was originally recognized in the field in 1952 as a sectored tassel. One fourth of the tassel was fully colored (reddish-purple) and the rest of the tassel had anthers with a colorless background and small reddish-purple stripes (Figure 1). Outcrosses were made with pollen from this tassel.

The genetic constitution of this original plant with reference to the a_1 locus was A_1/a_1^{dt}. A_1 is a full color allele and a_1^{dt} mutates to A_1 in the presence of *Dt*. *Dt*, however, was absent. It was verified by further tests that the A_1 allele and not the a_1^{dt} had mutated to a_1^m. The source of this particular A_1 allele was an unidentified midwestern inbred line.

The original mutable pattern was designated $a_1^{m(dense)}$ due to the dense appearance of the pattern of mutability (Figures 2b and 3a). Patterns of mutability are determined by the time (manifest in size of mutant area) and frequency (manifest in number of mutant areas) of mutation events (Table 1). The dense pattern type is intermediate in timing and possesses a high rate of mutability. From this original dense pattern other patterns showing a change in frequency (high or low) and timing (late or early) of the mutation events were recovered (Figure 3). Some of the kernels showing the diverse expression possible with a_1^m are shown in Figure 2. These patterns are based on inherent differences in the allele itself and are not due to modifiers, since in successive outcrosses (5–6 generations), a particular pattern shows a consistency in pattern type (Figure 3).

In addition to the above mentioned exceptional pattern types, a number of colorless kernels also appeared. Although all colorless kernels appear similar, genetic differences were demonstrated with appropriate tests. The origin of these colorless kernels as well as those showing pattern changes is not necessarily associated with a meiotic event since frequent somatic changes are observed (Figure 4). Germinal mutations to purple and intermediate pigmentation levels (pales) also occur.

The pales arising from a_1^m are generally lighter in color than the A^d pale alleles described by LAUGHNAN (1952). Although they are members of the same allelic series at the A_1 locus, they seem to have a different origin. The A^d pale allele

FIGURE 1.—Colored stripes on a colorless background of anthers of mutable plants showing the mutation from a_1^m to A_1.

arises as a result of a crossover event (LAUGHNAN 1960). The pales of the a_1^m series are observed as somatic mutation and the germinal mutations are not necessarily associated with crossover events. Studies on the interaction of these allelic forms are in progress.

In order to expedite the detection and isolation of changes in the parent pattern, the mutable allele was almost universally carried in the heterozygous form with $a_1^{dt}sh_2$ and testcrossed by this parent. These two genes, a_1^{dt}, which is colorless and mutates in the presence of *Dt* and sh_2, which results in a shrunken kernel, are .25 units apart. Thus, the a_1^{dt} allele is inherited with the sh_2 allele except for crossovers (1/800) appearing as colorless nonshrunken ($a_1^{dt}Sh_2$).

In the following crosses, reference to mutable or colorless kernels denotes only the nonshrunken (Sh_2) kernels unless otherwise specified.

TABLE 1

Designation of symbols

$a_1^{m(dense)}$	= a very dense mutable pattern: mutates from colorless to color (purple or red, depending on *pr*)
Derivative patterns	*first letter* designates the timing of the mutation event f = a *fine* pattern, late occurring mutation e = early occurring i = intermediate *second letter* designates rate h = high m = medium l = low for example: $a_1^{m(f.m.)}$—late occurring, medium frequency mutation
$a_1^{m(r)}$	= colorless form that responds to *En*
$a_1^{m(nr)}$	= colorless form that does not respond to *En*
En	= mutable component necessary for mutability to occur: appears at a_1 locus and by transposition becomes located at an independent position
I	= mutable component adjacent to mutable locus which is hypothesized to inhibit gene activity
I–En	= two components of a specific system (contrast to other systems such as a_1–*Dt*, *Spm*, *Ac–Ds*)
Independent *En*—*En*	in a chromosome position independent of the a_1 *locus* (with the $a_1^{m(r)}$ allele gives typical one mutable to one nonmutable when in heterozygous state)
Autonomous *En*—*En*	at a_1 locus—(in backcross tests gives almost all mutables due to its presence adjacent to locus)
a_1^{dt}	—colorless aleurone mutates to A_1 (colored aleurone) in presence of *Dt*; in text, referred to as a_1
Testcross	—cross of the heterozygous type by the homozygous recessive

RESULTS AND DISCUSSION

The mutable allele here described has a dense pattern which is characterized by a very high rate of mutability (Figures 2b and 3a). Three types of changes are recovered in the nonshrunken (Sh_2) progeny of testcrosses of this allele—$a_1^{m(dense)}Sh_2/a_1^{dt}sh_2 \times a_1^{dt}sh_2/a_1^{dt}sh_2$. These include kernel changes to fully colored and colorless, in addition to many permanent and heritable pattern alterations. An array of pattern types is recognized; these are due to differences in timing and frequency of mutation events (Figures 2 and 3 and Table 1). In addition to these pattern exceptions, colorless types appeared in a high frequency (Figure 3a). Two representative families that arose from testcrosses of this dense allele (out of more than 300 families) tested over a 5-year period are shown in Table 2.

Differences in the stability of individually isolated dense-mutable alleles can be observed between families 1958 492 and 1958 500 listed in Table 2. Although these two families represent pattern types that are phenotypically similar and

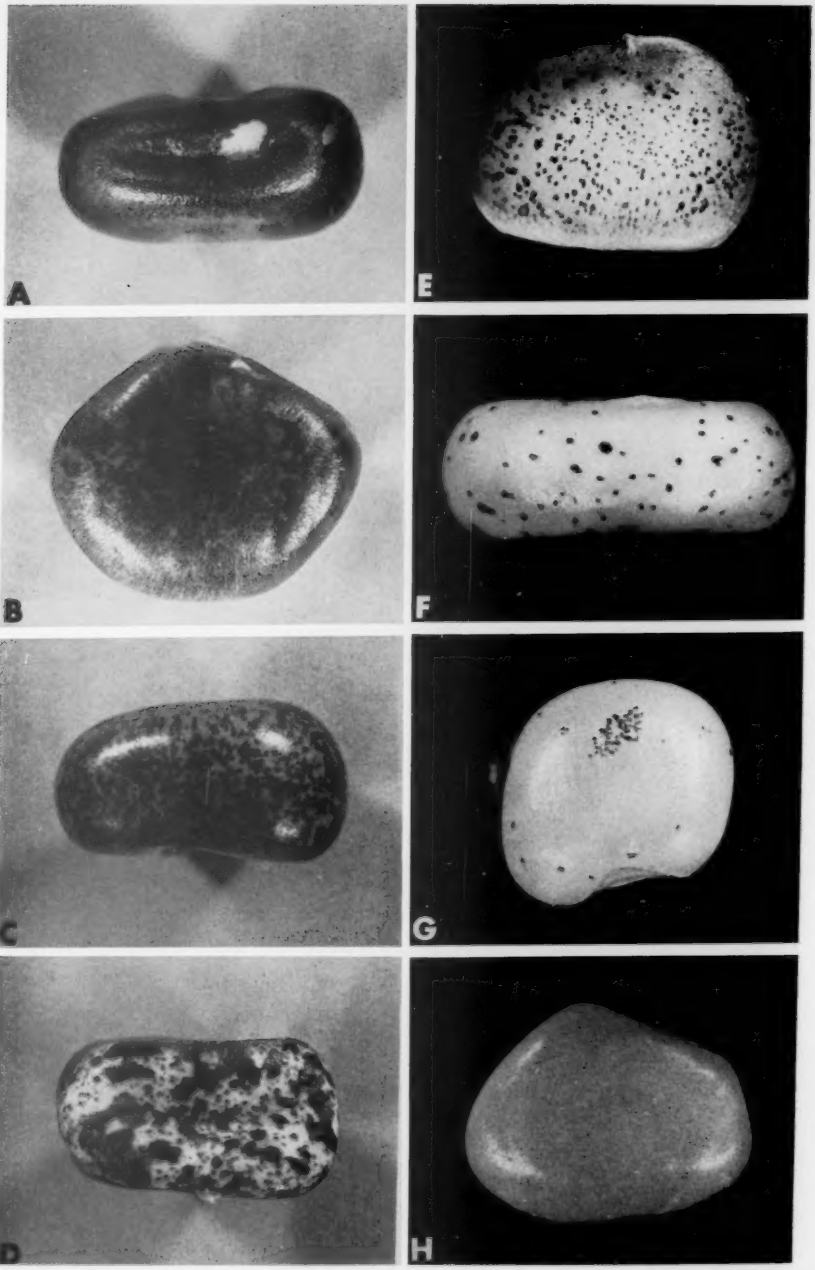

FIGURE 2.—Various mutable patterns of a_1^m (see Table 1). (A) very dense—universally recognized by the colorless sector; (B) dense; (C) f.h.; (D) i.m.; (E) f.m.; (F) f.l.; (G) low type with a reversion to f.h.; (H) colorless—$a_1^{m(r)}$ or $a_1^{m(nr)}$ depending on its response to *En*.

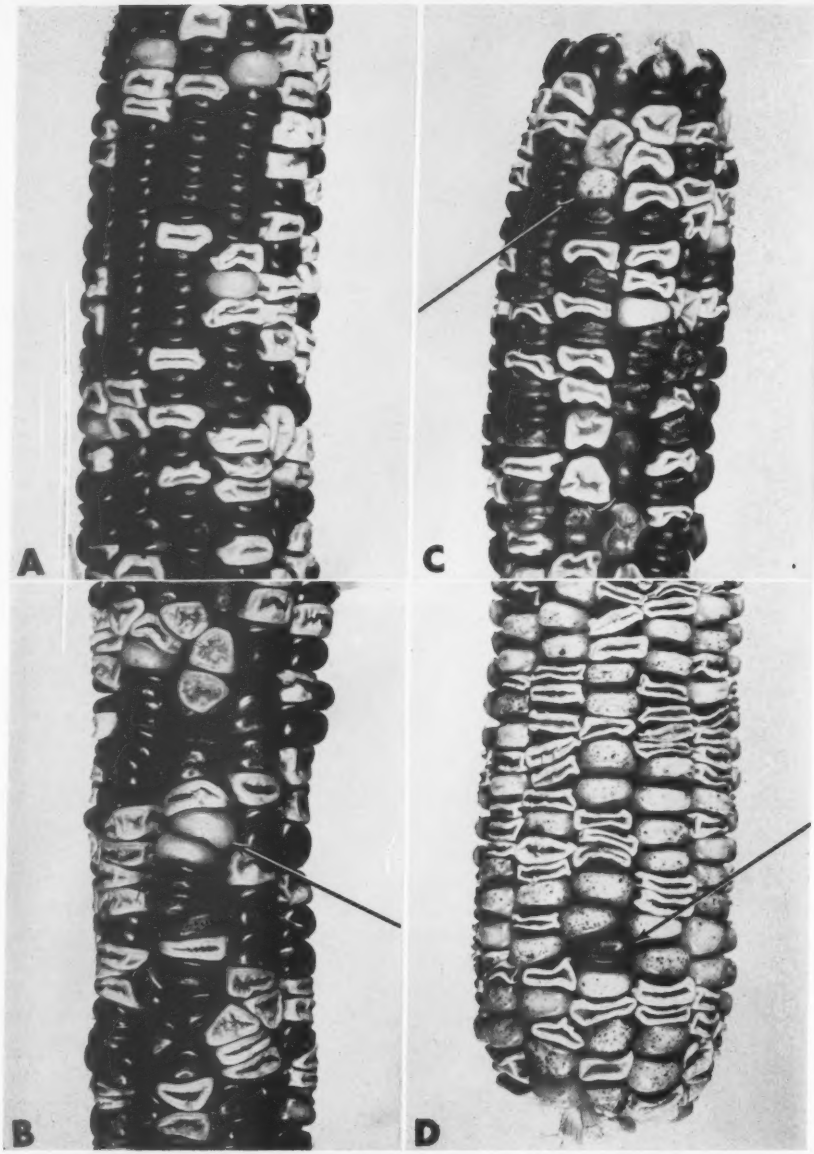

FIGURE 3.—The origin of pattern differences. These exceptions to the parental pattern are heritable; arrows indicate some of the exceptions. (A) dense with colorless exceptions. These exceptions are almost universally $a_1^{m(nr)}$ type. (B) $a_1^{m(f.h.)}$ with 4 $a_1^{m(f.l.)}$ exceptions (C) dense with a coarse exception. (D) a dense derivative in $a_1^{m(f.m.)}$.

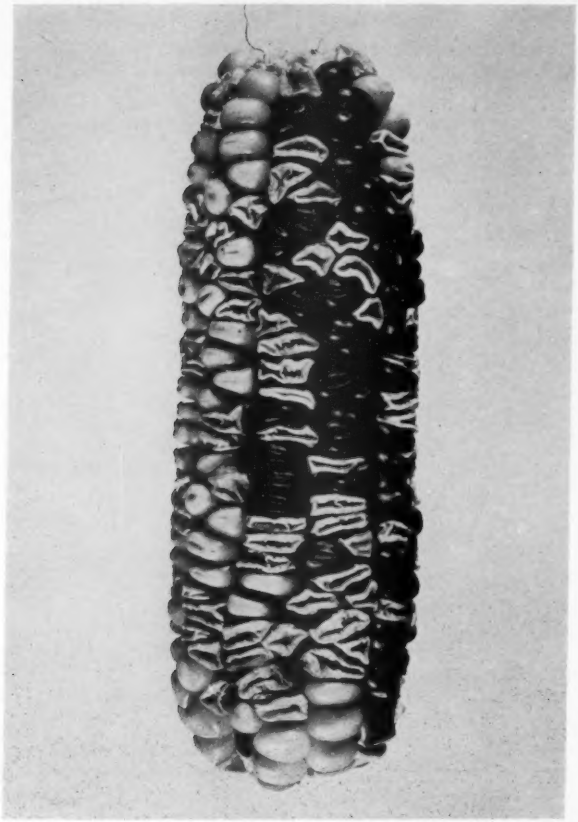

FIGURE 4.—Somatic change from a dense type mutable, $a_1^{m(dense)}$, to a stable, nonmutating allele $a_1^{m(nr)}$. The cross $a_1^{m(dense)}Sh_2/a_1^{dt}sh_2 \times a_1^{dt}sh_2/a_1^{dt}sh_2$. The dots seen on some of the kernels are the result of the action of *Dt* on the a_1^{dt} allele.

have a common origin, differences are nevertheless observed in the frequency of occurrence of colorless types arising from testcrosses. In 1958 492, the frequency of colorless types averaged 14.54 percent, while in 1958 500, it was 8.29 percent. Although there is variation in the frequency of colorless types among individual plants of a family, a common trend within a family does exist. Such a difference between families indicates that a similarity in pattern does not necessarily imply a similarity in mutational behavior.

Mutability is autonomously controlled in these original mutable alleles; i.e., in outcrosses and testcrosses most of the derived nonshrunken (Sh_2) progeny are mutable (Tables 2 and 3). This means that the mutability controller is closely

TABLE 2

The occurrence of colorless types and pattern exceptions in testcross progenies of individual ears from two dense mutable families (1958 492 and 500) arising from the cross* $a_1^{m(dense)}Sh_2/a_1sh_2 \times a_1sh_2/a_1sh_2$

1958 Source	Dense mutable	Colorless exceptions	Pattern exceptions	Total	Percent colorless exceptions
492–1	114	16	1 f.m.	131	12.21
–2	64	11	2 pale	77	14.28
–3	166	15	0	181	8.29
–4	80	18	0	98	18.37
–5	125	33	1 f.m.	159	20.75†
–6	179	43	0	222	19.37
–7	63	7	0	70	10.00
–8	150	21	0	171	12.28
–9	185	38	1 f.m.	224	16.96
–10	130	18	0	148	12.17
–11	133	23	0	156	14.74
–12	176	20	0	196	10.20
–13	76	18	0	94	19.15
–14	81	14	0	95	14.74
Average					14.54
500–1	135	2	0	137	1.46
–2	60	8	0	68	11.76
–3	152	26	1 f.m.	179	14.52
–4	111	10	1 f.m.	122	8.20
–5	98	7	0	106	6.60
–6	39	5	0	44	11.36
–7	149	18	0	167	10.78
–8	139	3	0	143	2.10
–9	81	7	1 f.m.	89	7.86
Average					8.29

* Only nonshrunken (Sh_2) kernels are considered.
† Used as 1959 56 (Table 3).

associated with the a_1 locus. This apparent inherent control of mutability by the locus itself is referred to as autonomous type control (autonomous *En*). From the autonomous type, an independent control of mutability is derived.

In this case the mutability controller is not closely associated with the mutant locus but is on another chromosome (as is true for *Dt* with reference to a_1^{dt} and *En* with *pg*) assorting independently of the mutant locus in question. Kernels lacking this controller would be colorless (designated $a_1^{m(r)}$ and discussed later). This type of control was found in some of the derivatives of the dense allele and is observed in testcrosses where one half of the nonshrunken (Sh_2) kernels are mutable and one half colorless (Table 4). Therefore from a controller that was originally located at or near the a_1 locus, an independently inherited controller arises.

The identity of the independent controller of mutability: Since from the auton-

TABLE 3

Tests for En *to verify* $a_1^{m(nr)}$ *types arising from the cross* $a_1^{m}Sh_2/a_1sh_2 \times a_1sh_2/a_1sh_2$*

Crossed × a_1sh_2	Pattern	Segregation a_1^m:cl	Percent cl	Tested as 1959	Carries *En*		Response of cl types to a known *En*
					In a_1sh_2 sibs	In cl	
7 99B–2	dense	39:11	22.0	3	0	+	—
7 101B–3	dense	119:15	11.2	4	0	+	—
7 155B–1	dense	171:18	9.5	13	—	+	0
7 160A–6	dense	125:28	18.3	14	0	+	0
7 166B	dense	150:28	15.7	15	0	+	0
7 171B	dense	69:21	23.3	16	0	0	—
7 177A	dense	89:14	13.6	17	0	+	0
8 410–3	dense	79:15	15.9	30	0	+	0
7 291–1	dense	60:34	36.17	31	—	+	—
7 291–5	dense	78:9	10.3	33	+	+	—
7 292–5	dense	77:39	33.6	34	+	+	0
7 294–13	dense	149:22	12.9	35	0	+	—
8 410–1	dense	166:12	6.7	43	+	+	—
8 418–3	dense sectoring	135:26	9.9	45	0	+	—
8 448–4	dense	75:16	17.6	51	0	+	0
8 470–3	dense	175:20	10.3	55	+	0	0
8 492–5	dense	125:33	20.9	56	0	0	—
8 492–9	dense	185:38	17.0	57	+	0	—
8 501–2	dense	101:30	22.9	58	+	0	—
8 448–6	dense	120:19	13.7	59	+	0	—
8 503–6	dense	107:36	25.2	60	+	0	—
8 502–6	dense	204:31	13.2	60	+	+	0

* Only nonshrunken *(Sh_2)* kernels considered in ratios. Mutability did not occur in any tests of the a_1sh_2/a_1sh_2 derivatives with the sib colorless types.
0 This test not completed.
— Negative test.
+ Positive test.
cl Colorless as in Figure 2h and 3a.

TABLE 4

Progeny of typical testcrosses of mutable plants carrying independent En *showing the segregation of mutable to colorless types: the cross* $a_1^{m(r)}Sh_2/a_1sh_2$ En/+ × a_1sh_2/a_1sh_2*

1959	No. of mutable	No. of colorless	Exceptions	Total	Percent cl
297–1	59	68	0	127	53.54
–2	55	53	0	108	49.07
–3	98	102	0	200	51.00
–4	110	103	0	213	48.36
306–1	64	59	0	123	47.97
–2	67	80	0	147	54.42
–3	60	69	0	129	45.73
–4	75	59	0	134	44.03
–5	65	59	0	124	47.58
–6	73	75	0	148	50.67
–7	58	63	0	121	52.07

* Only nonshrunken *(Sh_2)* kernels considered.

omous control of mutability an independent control is derived, the factor controlling mutability must be transposed from one location to another position in the genome. The independent controller-type is recognized by a testcross ($a_1^m Sh_2/a_1 sh_2 \times a_1 sh_2/a_1 sh_2$) ratio of 1 mutable nonshrunken:1 colorless nonshrunken (Table 4). The independent factor controlling mutability is present in the mutable (a_1^m) kernels and absent in the colorless ones. It would then follow that among the $a_1 sh_2/a_1 sh_2$ segregants from the above cross, one half possess the independent factor following random assortment. When a number of these $a_1 sh_2/a_1 sh_2$ segregants from testcrosses of independent types are tested against colorless kernels—$a_1^{m(r)}$—that are known to respond in the presence of the independent factor, mutability results on the ears in approximately half of the crosses. This indicates the existence in these $a_1 sh_2/a_1 sh_2$ segregants of a controlling factor assorting independently.

Is this independent controller identical to the Enhancer (*En*) of the *pg* system (PETERSON 1960a)? It may be recalled that *En* causes the pale green stable allele to become mutable. The following evidence demonstrates that the controller of a_1^m mutability is *En*.

To demonstrate the relationship between a_1^m and pg^m, it is necessary to prove that the independent controller which causes the derived colorless form to become mutable also causes the pale green stable to become mutable. (The symbols associated with the particular alleles in the cross are described in Table 1.)

The results show that *pg* mutability is associated with mutability of the colorless allele ($a_1^{m(r)}$—Table 1) and conversely the *pg* allele does not become mutable when the $a_1^{m(r)}$ allele remains immutable (Table 5). Since the factor controlling *pg* mutability is *En*, the factor causing a_1^m mutability must also be *En*. These correlative tests are a confirmation that the new mutable does indeed belong to the *En* system. In retrospect, one can say that a previously dominant allele (A_1) is changed to a mutable form, a_1^m, by the transposition of mutable components from the *Pg* locus to the A_1 locus. These two mutables respond to the same specific elements (*I* and *En*). They have no relation to other systems such as *Ac–Ds* or a_1–*Dt*. The $a_1^{m(r)}$ allele which becomes mutable in the presence of *En* does not respond to *Dt* nor to *Ac*; in the presence of *Dt* and without *En*, the kernels are stable and colorless. Demonstration of the relationship between a_1^m and pg^m seems proof that the transposition of the elements of the *En* system, *I* and *En*, lead to the origin of a new mutable.

Autonomous mutability: It was originally hypothesized that the dense allele was autonomous. In view of the high frequency of colorless types, consideration should be given to the possibility that this allele is not autonomous but that these colorless types arise from the independent assortment of more than one *En*. It may be recalled that the presence of *En* is necessary for the colorless form to become mutable and, in addition, it is known that more than one *En* may exist in the genome. If, for example, three *En* elements were assorting independently in a testcross ($En_1/+, En_2/+, En_3/+$), one eighth of the resulting progeny would lack *En* and be nonmutable. Such a rate (12 percent) is very similar to the rate

TABLE 5

The test of similarity in response of $a_1^{m(r)}$ *and* pg^s *to* En. *The cross*
$+/pg^sA_1A_1 \times Pg/Pg\ a_1sh_2/a_1sh_2$
Two kinds of F_1 plants.*
One half $Pg/pg^s\ A_1Sh_2/a_1sh_2$ no mutable factor.
One half $Pg/pg^s\ A_1Sh_2/a_1sh_2$ with mutable factor.
F_1 selfed and tested on $a_1^{m(r)}$.

Source	Total no. of ears	Gave a_1 mutable in progeny of ⊗		Did not give a_1 mutable in progeny of ⊗	
		Segregates pg^m and pg^s	Segregates Only pg^s	Segregates pg^m and pg^s	Segregates Only pg^s
1958	.	.	.	.	.
1370	8	4	0	0	4
1371	4	1	0	0	3
1372	3	2	1†	0	0
1373	12	8	0	0	4
1959	..	.	.	.	.
1040	20	9	0	0	11
		24	1	0	22

* Only the F_1 plants with pg^s are shown, since only these are relevant to the experiment.
† Only exception to simultaneous induction of instability in pg^s and $a_1^{m(r)}$. In all other cases, the occurrence of pg^m is correlated with a_1 mutability.
⊗ Self-pollination.

of stables appearing among the testcross progeny of the autonomous alleles (Tables 2 and 3). Crosses were made to try to resolve this problem.

The autonomous alleles in crosses have always been carried as heterozygotes with a^{dt} and sh_2 on the homologous chromosome in the form $a_1^{m(dense)}Sh_2/a_1^{dt}sh_2$. It was previously noted that a_1 and sh_2 are so closely linked that colorless crossovers (a_1Sh_2) appear at the rate of 1/800 in testcrosses. Under the assumption that the high rate of colorless forms is based on many *En* assorting independently, one could then test for the presence of *En* in the a_1sh_2 segregants. The presence of *En* is detected by testing the a_1sh_2 segregants as well as the colorless exceptional types originating from the cross $a_1^{m(dense)}Sh_2/a_1sh_2 \times a_1sh_2/a_1sh_2$. This was done by crossing these two types, a_1sh_2 segregants and colorless forms, by $a_1^{m(r)}$. If mutability is observed in these tests, *En* must be present. If only colorless forms appear, then *En* is absent. Progenies from the two crosses involving this a_1sh_2 and colorless Sh_2 segregants were examined.

In one type of cross, that between the shrunken segregants and their colorless sibs from testcrosses of assorted mutable alleles (Table 2), mutable progeny were not obtained. This indicates that the colorless types were not due to the segregation of *En* and must arise from mutation events. In crosses of these same a_1sh_2 segregants with a known $a_1^{m(r)}$ mutability resulted, but mutability also resulted when the colorless sibs were tested in this same manner; therefore *En* must be present. These colorless forms were then tested by crossing them with a known *En* stock. In these tests, *En* again was not effective since mutability did not result.

It is obvious from these tests that the colorless forms are not mutable in the presence of *En*. Such results indicate that these colorless forms derived as exceptional types from testcrosses of the dense mutable alleles are nonresponsive to *En* and therefore are designated $a_1^{m(nr)}$. This allele would correspond to a^{dl} originating from a_1^{dt} in *Dt* stocks (RHOADES 1941). A similar nonmutable allele was found in the mutable pericarp series. This stable type possesses the mutability controller, Modulator, at the *P* locus (BRINK 1958a). In the *Ac–Ds* system, similar nonresponding *bz* derivatives are obtained (MCCLINTOCK 1956).

It has been shown that the high rate of incidence of colorless forms among the testcrosses of autonomous alleles is due to mutation. Although *En* is carried in multiple in these stocks, the colorless forms do not result from the independent assortment of *En* since they are not mutable when *En* is present.

In addition, this experiment has uncovered a second type of colorless allele, $a_1^{m(nr)}$, which does not respond in the presence of *En*. This colorless allele, $a_1^{m(nr)}$, is differentiated from the other colorless allele, $a_1^{m(r)}$ that does respond in the presence of *En*. (The superscript r = responds and nr = does not respond to *En*). These two alleles are then isoalleles in the original sense since they are phenotypically alike and can be differentiated only by more definitive tests (in this case, response to *En*). These are similar to isoalleles described in Neurospora (HOROWITZ and FLING 1953; WEBBER 1960) and in Drosophila (STERN and SCHAEFFER 1943) which are distinguished by temperature sensitivity of the gene product, histidine biosynthesis and by mutation sequence, respectively. There have been many reports of wild-type isoalleles which are distinguishable mainly on their differential rate of mutability (LEFEVRE 1955; GREEN 1959). A similar difference in mutability of seemingly identical alleles is evident among a number of $a_1^{m(dense)}$ alleles in their rate of change from mutable to colorless forms (Tables 2 and 3). Many of these cases of isoalleles reaffirm the notion that phenotypic expression is not the only indicator of the potential expression of the gene in question. This suggests a wide latitude in genic alterations that are subliminal and do not result in phenotypic differences. These are therefore not readily ascertained.

In a sense the origin of the $a_1^{m(nr)}$ allele reminds one of BRINK's paramutation event in that this colorless form is a permanent change caused by the presence of another element, *En*. It is distinguished from a paramutation event (BRINK 1958b) in that the change is not universal and not necessarily invariable since changes can result in altered pattern expressions, in addition to the nonmutable colorless forms. It might, however, be a form of the same kind of event.

SUMMARY

A new mutable gene at the a_1 locus (a_1^m) originated from an A_1 allele in a pale green mutable stock.

The mutability of the original a_1^m allele is autonomously controlled, i.e., the control of mutability is intimately associated with the a_1 locus. From this type an independent controller located independently of the a_1 locus has been isolated.

In addition it was found that the mutability controller of a_1^m also causes *pg* to be mutable. From these and other results, the a_1^m controller must be identical to *En* (Enhancer) of the *pg* system. This finding is indicative of a transposition event—the transfer of the mutable components of *pg* to the a_1 locus.

A wide range of patterns is derived from the original mutable pattern (dense) and these include various pigmentation types. In addition colorless types arise, some of which respond to *En*. The nonresponding type occurs at a high rate and represents an irreversible loss of color potential at this locus.

The relations between different alleles of a mutable series and isoalleles in general and paramutation events are discussed.

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LETHAL EQUIVALENTS IN DOMESTIC ANIMALS[1]

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MORTON, CROW and MULLER (1956) presented a method for estimating the average number of lethal equivalents per individual. A lethal equivalent corresponds to a lethal gene with 100 percent probability of causing death, or to two genes with an average 50 percent probability of causing death, or to three genes with one third probability each of causing death, etc. The average number of lethal equivalents may be estimated indirectly through the observed excess of detrimental effects in the offspring of consanguineous matings, as compared with nonconsanguineous ones. The authors cited arrived at the formula:

$$-log_e S = A + Bf,$$

where f is the inbreeding coefficient, S is the expected fraction of survivors, B is *practically* the mean number of lethal equivalents per gamete (actually the mean number of lethal equivalents is B plus the genetical component of A), and A is the detrimental effects due to the lethal equivalents in panmitic populations plus environmentally caused deaths. The method of analysis proposed, with about one or two iterations leads to minimum variance solution.

We decided to examine the literature for data on domestic animals on hatching, abortion and early death correlated with inbreeding coefficients. Such data were found for chickens, swine and cattle, and the results of calculations regarding lethal equivalents are presented here.

Chickens

The data here analyzed were obtained from publications by JULL (1929), BERNIER, TAYLOR and GUNNS (1951), MACLAURY and NORDSKOG (1956) and WATERS (1945).

These authors worked with two breeds, Barred Plymouth Rocks (Tables 1, 2 and 3) and Single Comb White Leghorns (Tables 4, 5, 6, 7, and 8). The data for

TABLE 1

Hatchability and inbreeding in Barred Plymouth Rocks (Data from Table 1 of JULL *1929)*

N	f	S	$-log_e S$
41	0.000	0.745	0.294
19	0.125	0.452	0.794
30	0.250	0.329	1.112

N = number of families; f = inbreding coefficient; S = fraction of survivors.

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TABLE 2

Hatchability with different degrees of inbreeding in Barred Plymouth Rocks
(Data from Table 2 of JULL *1929)*

N	f	S	$-\log_e S$
21	0.00000	0.761	0.273
17	0.12500	0.473	0.749
30	0.25505	0.419	0.870
25	0.33505	0.282	1.266

TABLE 3

Hatchability under different degrees of inbreeding in Barred Plymouth Rocks
(Data from Table 6, JULL *1929)*

N	f	S	$-\log_e S$
17	0.125	0.473	0.749
12	0.250	0.401	0.914
10	0.375	0.211	1.156

TABLE 4

Hatchability in families with different degrees of inbreeding in Single Comb White Leghorns
(Data from Table 3, JULL *1929)*

N	f	S	$-\log_e S$
43	0.00000	0.820	0.198
30	0.12500	0.608	0.498
46	0.25505	0.573	0.557
53	0.33505	0.475	0.744
6	0.49505	0.476	0.742

TABLE 5

Hatchability in families of White Leghorns with different degrees of inbreeding
(Data from Table 5, JULL *1929)*

N	f	S	$-\log_e S$
10	0.0000	0.810	0.211
6	0.1250	0.699	0.358
6	0.2500	0.625	0.470
5	0.2594	0.617	0.483
5	0.3750	0.463	0.770
8	0.3437	0.451	0.796
5	0.5000	0.434	0.835

TABLE 6

Hatchability in families of White Leghorns with different inbreeding coefficients (Data from Table 7, JULL *1929)*

N	f	S	$-\log_e S$
20	0.125	0.584	0.538
17	0.250	0.566	0.569
12	0.375	0.520	0.654
5	0.500	0.434	0.835

TABLE 7

Data on hatchability in 1940–1941, in White Leghorns, in relation to inbreeding coefficients (Data from Table 15 of BERNIER, TAYLOR and GUNNS *1951. Only lines with known coefficients of inbreeding were used)*

Number of eggs	f	S	$-\log_e S$
117	0.0000	0.8364	0.179
165	0.0000	0.7216	0.328
230	0.0000	0.8249	0.192
135	0.1250	0.7289	0.316
126	0.2057	0.6270	0.467
65	0.3375	0.6861	0.377

Barred Plymouth Rocks are presented in Tables 1, 2 and 3. The value of N in these tables does not exactly correspond to that of MORTON and collaborators, i.e., it is not the number of events but the number of families of birds or of fertile eggs. Hence, we did not use their formula for computing variance but rather the conventional method described by SNEDECOR (1948) and WILLIAMS (1959).

The estimation of B, of the variance of B, of A and of B/A gave for Barred Plymouth Rocks the results shown in Table 9. The value of B in Table 2 was statistically different from zero at the five percent level. The other differences were close to the five percent level but did not reach it. The reason for this is that our N is the number of families rather than the number of fertile eggs and, therefore, is low. Furthermore, our statistical analysis in all cases was conservative, the degrees of freedom being based on the inbreeding level. If instead of the level of inbreeding the number of families were used, all three B values would be significantly different from zero and also different from each other at below the 0.1 percent level. However, considering that the birds of Table 1, 2 and 3 belong to the same flock (for instance, Table 2 contains the same families as Table 1 under continued inbreeding for two more years), an analysis showing such enormous statistical differences might be suspect. Hence, a less efficient type of analysis was used. Employing for it the number of families, high statistical precision that might distinguish groups that actually would not be distinct might be obtained. Part of this dilemma happens to be due to the fact that the data were

TABLE 8

Effect of inbreeding of White Leghorns selected for high hatchability (Data from Figure 2 of WATERS *1945)*

N	f	S	$-\log_e S$
4	0.00	0.89	0.116
2	0.00	0.92	0.083
4	0.00	0.96	0.041
3	0.00	0.92	0.083
2	0.00	0.91	0.094
2	0.00	0.91	0.094
3	0.00	0.94	0.062
3	0.00	0.79	0.236
3	0.00	0.97	0.030
2	0.00	0.87	0.134
2	0.00	0.90	0.105
1	0.00	0.96	0.041
2	0.00	0.98	0.020
5	0.00	0.87	0.139
3	0.00	0.90	0.105
6	0.20	0.78	0.248
10	0.25	0.49	0.713
8	0.25	0.83	0.186
9	0.29	0.75	0.288
17	0.29	0.72	0.328
10	0.29	0.84	0.174
12	0.33	0.60	0.511
12	0.33	0.78	0.248
12	0.38	0.70	0.357
9	0.38	0.49	0.713
10	0.38	0.50	0.693
8	0.38	0.79	0.236
16	0.38	0.72	0.328
9	0.59	0.51	0.673
16	0.59	0.65	0.431

TABLE 9

Results of the analysis of Tables 1, 2 and 3

Table	B	Variance of B	A	B/A
1	3.400	0.105	0.299	11.374
2	2.673	0.155	0.284	9.402
3	1.527	0.034	0.552	2.766

not our own but obtained from the literature. Hence, the numbers of eggs per family used to obtain the observed value of S are not known to us, and we could only compute the mean survival for groups having given f values.

A covariance analysis was made of the three different B values (Table 10).

TABLE 10

Covariance analysis for data on Barred Plymouth Rocks

Source of variation	D. F.	Sum of squares	Mean square	F
Common regression	1	23.193	23.193	....
(Heterogeneity)	(2)	(0.666)	0.333	2.061 (n.s.)
Individual regression	3	23.859	....	....
Residual error	(4)	(0.646)	0.162	....
Total within the series	7	24.505	....	F at 5% = 6.94

It shows that the three values of B are not statistically different from each other; hence, the data could be pooled and only one value for B calculated for the total. This value is found to be 2.840, differing from zero at less than the 0.1 percent level. Therefore, the number of lethal equivalents acting in the embryonic life of this breed of chickens ($2B$) is estimated to be 5.68.

According to CROW's (1958) model, the index B/A indicates if the genetic load is mutational (when this ratio is high, provided there is no multiple allelism) or if it is due to heterotic genes (low values). The value B/A for our sample is 9.55 which we consider to be low.

The data for Single Comb White Leghorns are presented in Tables 4, 5, 6, 7 and 8. The estimates of B, of the variance of B, of A and of B/A for these data gave the results tabulated in Table 11. The covariance analysis in Table 12

TABLE 11

Result of the analysis of Tables 4 to 8

Tables	B	Variance of B	A	B/A
4	1.486**	0.041	0.213	6.967
5	1.343***	0.022	0.202	6.654
6	0.672	0.037	0.429	1.567
7	0.754	0.102	0.216	3.493
8	0.753***	0.009	0.067	11.315

** = Significant 1% level.
*** = Significant 0.1% level.

TABLE 12

Covariance analysis for data on Single Comb White Leghorns

Source of variation	D. F.	Sum of squares	Mean square	F
Common regression	1	47.798	....	....
(Heterogeneity)	(4)	3.147	0.787	1.634 (n.s.)
Individual regression	5	51.651	....	....
Residual error	(42)	20.212	0.481	....
Total within the series	47	71.158	....	....

was made to see if these data are homogeneous since they were obtained from three different authors. The data in Table 8 were calculated from a graph of WATERS (1945).

As one may see from the F test in Table 12, there are no statistical differences among the five classes of data. Hence, they can be added together and only one value of B calculated. This is found to be 0.847, with a variance equal to 0.008. The lethal equivalents per bird ($2B$) affecting the embryonic life of White Leghorns are therefore 1.69. This number was so different from that found for Barred Plymouth Rocks that an analysis of comparisons between groups was made (see Table 13). The t test was significant, so that the B for Leghorns must be considered different from the B for Barred Plymouth Rocks below the 0.1 percent level.

TABLE 13

Comparison of B *coefficients of Barred Plymouth Rocks and Single Comb White Leghorns*

Races	Sxx	Sxy	Syy	B	$(Sxy)^2/Sxx$	$S_e=Syy-(Sxy)/Sxx$	D.F.	$1/Sxx$
Plymouth	2.875	8.165	24.505	2.840	23.193	1.312	6	0.348
Leghorn	66.674	56.452	71.158	0.847	47.798	23.359	46	0.015
Total	69.549	64.618	95.663	1.994*	70.991	24.671	52	0.363

* Difference.

x represents here f (the inbreeding coefficients) and y represents $-\log_e S$.

$$t = \frac{1.994}{0.416} = 4.793$$

$$t_{(40;\ 0.1\%)} = 3.551$$

In addition to these data (Tables 1 to 8), which enabled us to estimate the average number of lethal equivalents affecting the embryonic life of these two breeds, we found in the literature one other class of data that permitted further conclusions to be arrived at. MACLAURY and NORDSKOG (1956) studying the effects of inbreeding on mortality in Single Comb White Leghorns summarized their data in a table which corresponds to our Table 14. Instead of the limits of the classes

TABLE 14

Distribution of mortality in relation to inbreeding coefficients in three different age levels (Data from Table 1 of MACLAURY and NORDSKOG *1956)*

f	Survivors from hatching to 8 weeks	Survivors from 8 weeks to 6 months	Survivors from ±6 months to 18 months
0.05	0.836	0.837	0.743
0.15	0.880	0.901	0.663
0.25	0.823	0.866	0.689
0.35	0.856	0.782	0.610
0.45	0.741	0.761	0.536
0.55	0.816	0.796	0.494
0.65	0.758	0.762	0.459
0.75	0.701	0.753	0.433
0.85	0.662	0.771	0.414

of inbreeding coefficients, we used the center of the classes (e.g., instead of 0.10 to 0.19, we used 0.15), and instead of giving the percentage mortality, the percentage of survivors is shown in the table. In this case, because of the lack of information on the number of birds at each level of inbreeding, we could only calculate the unweighted regression of f on $-log_e S$ for the three columns. For the first, B (Table 14) is 0.304 (variance of $B = 0.0045$, $A = 0.108$, $B/A = 2.819$). Hence, the average number of lethal equivalents ($2B$) per bird, affecting the first eight weeks of life is 0.608. For the second column, B is 0.193 (variance of $B = 0.003$, $A = 0.139$, $B/A = 1.32$) so that the average number of lethal equivalents affecting these birds from eight weeks to six months is 0.366. The third column gave a B value of 0.773 (variance of $B = 0.0025$, $A = 0.252$, $B/A = 3.064$). Therefore the average number of lethal equivalents per bird affecting the first year of laying (six to 18 months) is 1.546. The three B values are different from zero at one percent, five percent and 0.1 percent level, respectively.

Discussion: Adding up the values of B which affect the embryonic life, the first eight weeks, eight weeks to six months of age, and six months to 18 months of age, we obtain for White Leghorns a total B of 2.103. Each individual, therefore, has an average of at least 4.206 deleterious gene equivalents.

The fact that the value of B (affecting embryonic life) for Barred Plymouth Rocks (2.84) differs statistically ($P < 0.0001$) from the value for Leghorns (0.85) is not surprising. In fact, different number of lethals have been found in different strains of other organisms. The frequency of lethal and semilethal second and third chromosomes of *Drosophila willistoni* found in populations from Florida, Cuba, and several regions of Brazil are not uniform; that is, several of these populations have frequencies of lethal equivalents that are statistically different from others (PAVAN, CORDEIRO, DOBZHANSKY, DOBZHANSKY, MALOGOLOWKIN, SPASSKY and WEDEL 1951; CORDEIRO 1952; TOWNSEND 1952). In *Drosophila pseudoobscura*, two California races have half the percentage of lethals and semilethals (third chromosome) that a race from Mexico and Guatemala has (16.5 and 13.9 percent against 30.0 percent) (DOBZHANSKY 1951).

Recently FREIRE-MAIA, FREIRE-MAIA, and QUELCE-SALGADO (1960) studied a human population in interior Brazil and found the average number of lethal equivalents below one for Caucasoids, and between nine and ten in Negroes, both groups living in the same environment (rural area). Therefore, the difference between Single Comb White Leghorns and Barred Plymouth Rocks obtained by this method is similar to those obtained by the same method for different human races, or to the ones obtained by a more direct method in populations of two Drosophila species.

Another interesting result was the very low values of B/A—an indication that the genes in question are overdominant. In case there are many alleles per locus this conclusion is strengthened. In fact, at least four multiple allelic loci are known in chickens: *T*, *Po*, with three alleles each (HUTT 1949), and the two blood group loci, *A* with nine alleles (BRILES, MCGIBBON and IRWIN 1950) and *B* with 21 alleles (BRILES, ALLEN and MILLER 1957). Furthermore, there are

TABLE 15

Estimation of lethal equivalents disregarding the figures for inbreeding coefficients equal to zero (f=0), compared to these based on all data

Tables	B_0 with $f=0$	B_1 without $f=0$	B_0-B_1
2	2.673	2.081	0.592
4	1.460	0.971	0.489
5	1.343	1.513	−0.170
7	0.754	0.370	0.384
8	0.753	0.701	0.052

abundant data revealing the existence of overdominant genes in fowls. BRILES (1954) showed that the hatchability of fertile eggs of three inbred lines of White Leghorn produced by matings leading to 0, 50, 75 and 100 percent heterozygosity of three *B* alleles was 46, 62, 71 and 78, respectively. This indicates that a heterozygous embryo in that experiment had 59 percent more probability of hatching than a homozygous one. The average egg production of heterozygous classes within each line was from nine to 30 percent greater than that of the homozygous ones. COCK (1956), studying the segregation of hypostatic color genes within inbred lines of chickens, found that cryptic plumage color genes were segregating in a frequency higher than could be expected by chance, suggesting a heterotic effect for this gene. Other data pointing to similar conclusions are to be found in the report of SHULTZ and BRILES (1953) and in GILMOUR's review (1959).

BORISSENKO (1945) observed that in eggs of an inbred stock of White Leghorns the relative amount of thin albumen showed a tendency to decrease with continued inbreeding; solids in the thin albumen diminished; the phosphorus content of thin and thick albumen declined (cited by LANDAUER 1951). The influence of the inbreeding of the mother on hatchability can be roughly measured comparing the estimates of lethal equivalents calculated with the values for $f = 0$, and the estimates of lethal equivalents leaving such values out. If the inbreeding of the mother has any influence on the hatchability of the offspring, we should obtain a smaller estimated value for *B* when all mothers are inbred. In Table 15 these values were tabulated: B_0 is the estimate (already calculated in Tables 2, 4, 5, 7 and 8), which includes values for $f = 0$; B_1 is the estimate made from the same data disregarding the figures for $f = 0$. As it may be seen, Table 15 suggests that we have an overestimate of *B*; a statistical analysis of the differences B_0-B_1 produced a t equal to 2.04, which due to the small number of samples is not significant ($P \simeq 0.118$).

In conclusion for this section, it may be said that the data for chickens are in accordance with an idea of multiple allelic loci with overdominant effects of the deleterious genes.

Swine

The data for swine appear in Tables 16, 17, 18 and 19. They have been ob-

TABLE 16

Estimation of embryo survival in line N
(Data obtained from Table 1, N line, of HODGSON *1935)*

N^*	f	S†	$-\log_e S$
3	0.250000	0.808	0.213
3	0.375000	0.475	0.744
4	0.500000	0.625	0.470
6	0.593750	0.692	0.369
8	0.671875	0.492	0.710
5	0.734375	0.650	0.431
5	0.785156	0.483	0.729

* Number of litters.
† To calculate S, the greatest number of pigs born per litter (in this line it was 12) was considered as being 100 percent survival.

TABLE 17

Estimation of embryo survival in line M
(Data obtained from Table 1, line M, of HODGSON *1935)*

N^*	f	S†	$-\log_e S$
2	0.250000	0.868	0.142
6	0.375000	0.654	0.425
5	0.500000	0.523	0.648
7	0.593750	0.623	0.473
9	0.671875	0.438	0.824
2	0.734375	0.654	0.425

* N is the number of litters.
† To obtain S, the greatest number of pigs born per litter (in this line it was 13) was considered as being 100 percent of embryo survival.

TABLE 18

Estimation of embryo survival in line L
(Data from Table 1, line L of HODGSON *1935)*

N^*	f	S†	$-\log_e S$
2	0.250000	0.90	0.105
4	0.375000	0.70	0.357
6	0.500000	0.50	0.693
6	0.593750	0.70	0.357
4	0.671875	0.60	0.511

* N is the number of litters.
† To obtain S, the greatest number of pigs born per litter (in this line it was 10) was considered as being 100 percent of embryo survival.

tained from HODGSON (1935) who worked with Poland-China hogs. The first three of these tables are based on HODGSON's Table 1, which contains data for his L, M and N lines. These tables show the number of pigs born per litter. The maximum number per litter in each line was taken by us as being equivalent to

100 percent embryo development. It is assumed that litter size is a good measure of embryo survival. The B's for each line are shown in Table 20.

The F test (0.260) was nonsignificant; therefore, the data can be pooled and a simple B value and variance of B for all three lines obtained. These estimates were: $B = 0.816$; $A = 0.010$; variance of $B = 0.048$; $B/A = 78.279$. Therefore, in these lines of Poland-China hogs the average number of lethal equivalents ($2B$) per hog, affecting embryonic life, is 1.632. ($B = 0.816$ differs from zero at less than one percent level).

The results of this variance analysis allow us to estimate B for Table 19, containing data for all three lines and for one control. It is 0.200 and the variance of B is 0.070, while A is 0.261 and B/A is 0.764. This value of B does not differ significantly from zero.

From these results we can say that the average lethal equivalents per Poland-China hog affecting embryonic life is 2.032 (adding 1.632 to 0.400). Of course, it might be expected that breeds with greater or smaller values might be found.

TABLE 19

Early life survival of pigs born (Data obtained from Table 3 of HODGSON *1935)*

Lines	N	f	S	$-\log_e S$
Control	97	0.0000000	0.77	0.261
N*	79	0.7031250	0.73	0.315
M*	50	0.7031250	0.38	0.968
L†	66	0.6328125	0.67	0.401

* Average of two inbreds with five and six generations of inbreeding.
† Average of two inbreds with four and five generations of inbreeding.

TABLE 20

Summary of analysis in Tables 16, 17 and 18

Lines	B	Variance of B	A	B/A
L	1.134	0.256	—0.166	6.822
M	1.012	0.171	—0.035	—28.811
N	0.627	0.119	0.107	5.846

TABLE 21

Variance analysis of B *obtained for lines L, M and N*

Source of variation	D. F.	Sum of squares	Mean square	F
Common regression	1	3.114	....	....
Heterogeneity	(2)	(0.131)	0.066	0.260 (n.s.)
Individual regressions	3	3.245	....	....
Residual error	(12)	(3.036)	0.253	....
Total within the series	15	6.281	....	F at 5% = 3.88

Cattle

In the paper of REGAN, MEAD and GREGORY (1947) we found information on the relation of inbreeding to calf mortality in the Jersey and Holstein breeds of dairy cattle. This information is contained in Tables 22, 23, 24, 25 and 26, with Table 27 summarizing the B values. Those without an asterisk do not differ statistically from zero. Hence, the negative value of A for abortion in Jersey is due to random variation and has no biological meaning.

For the data presented in these tables the only value of B that differs from zero is the one for mortality to four months of age in the Jerseys. The Holsteins have a very low number of lethal equivalents per animal, near or equal to zero. However, Jersey animals have an average of 2.14 lethal equivalents affecting the first four months of postnatal life. Here, the ratio B/A does not give any reliable information.

TABLE 22

Relation of total mortality to the inbreeding coefficient in Jersey female calves to four months of age (Based on Table 2 of REGAN, MEAD *and* GREGORY *1947)*

N	f	S	$-\log_e S$
159	0.03	0.84	0.174
68	0.19	0.66	0.416
101	0.28	0.65	0.430
44	0.41	0.57	0.562

TABLE 23

Relation of total mortality to the inbreeding coefficient in Holstein female calves to four months of age (Based on Table 2 of REGAN, MEAD *and* GREGORY *1947)*

N	f	S	$-\log_e S$
46	0.02	0.87	0.139
18	0.15	0.89	0.116
18	0.27	0.79	0.236
19	0.39	0.90	0.105

TABLE 24

Relation of stillbirth to inbreeding coefficient in Jerseys (Based on Table 1 of REGAN, MEAD *and* GREGORY *1947)*

N	f	S	$-\log_e S$
340	0.02470	0.959	0.042
129	0.18085	0.969	0.031
222	0.27463	0.941	0.060
84	0.40023	0.915	0.088

N is the result of adding the number of males and females; f and S are weighted pooled values.

As a general conclusion, it may be said that the range of figures for lethal equivalents in domestic animals is not very different from the ones found in human populations. However, the ratio B/A indicates that overdominance is important in chickens and perhaps in swine, which does not seem to be the case either in human populations according to CROW (1958) or in cattle. The observed importance of overdominant loci which makes the main difference in the load of deleterious genes might be due to the long history of inbreeding in the domestic animals with a short reproductive life, through which ordinary lethals and sub-lethals may have been purged, so that, currently, the variance of fitness is largely determined by heterotic genes.

All tables with experimental data were obtained from papers by researchers

TABLE 25

Relation of stillbirth to inbreeding coefficient in Holsteins (Based on Table 1 of REGAN, MEAD *and* GREGORY *1947)*

N	f	S	$-\log_e S$
98	0.0200	0.980	0.021
41	0.1444	0.976	0.025
76	0.2651	0.987	0.013
43	0.3956	0.961	0.050

N represents the number of males and females; f and S are weighted values.

TABLE 26

Relation of abortions to inbreeding coefficient in Jerseys (Based on Table 1 of REGAN, MEAD *and* GREGORY *1947)*

N	f	S	$-\log_e S$
340	0.02470	0.971	0.015
129	0.18085	0.952	0.024
306	0.30910	0.992	0.003

The data represents males plus females. The fourth class with f equalling 0.40023 had to be pooled with the third because survival was one.

TABLE 27

Summary of the analysis made in Tables 22 to 26

Breed	Table	B	Variance of B	A	B/A
Holstein, abortion	..	....	....	....	....
Holstein, stillbirth	25	0.013	0.028	0.018	0.698
Holstein, early death	23	0.013	0.047	0.143	0.092
Jersey, abortion	26	0.044	0.001	—0.005	—9.299
Jersey, stillbirth	24	0.076	0.004	0.036	2.141
Jersey, early death	22	1.071*	0.026	0.147	7.295

* Significantly different from zero below the 5% level.

directly or indirectly involved in breeding programs. This introduces one extra distortion in the data, because in all cases treated here it is expected that the ancestors of the more highly inbred animals have also been subject to a higher selection pressure.

SUMMARY

The MORTON-CROW-MULLER method for determination of lethal equivalents was applied to domestic animals.

Data from literature on inbreeding in domestic animals were analyzed to determine the number of average lethal equivalents per animal. Chickens had 1.69 lethal equivalents affecting hatching in Single Comb White Leghorns and 5.68 in Barred Plymouth Rocks; these two genetical loads are statistically different (below the 0.1 percent level). Adding the numbers of lethal equivalents for hatchability for eight weeks of age, for eight weeks to six months of age, and for six months to 18 months of age, the total number of detrimentals for each Leghorn bird is 4.206. The ratio B/A agrees with other information on chickens, i.e., that some of the genes responsible for the deleterious load have overdominant effects, and some have more than two alleles per locus.

Poland-China hogs have 1.632 lethal equivalents per animal affecting embryonic life and 0.400 affecting early life.

Jersey and Holstein cattle have very low average lethal equivalents. In fact, the values obtained for Holsteins do not differ statistically from zero. The only significant value found was for genes affecting viability from birth to four months in Jerseys; in this case the average number lethal equivalents is 2.14.

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INDUCED MUTATIONS AT THE V^{by} LOCUS OF TRIFOLIUM REPENS. II. REDUCTION BELOW THE ADDITIVE BASE LINE BY FRACTIONATED DOSES OF GAMMA RADIATION

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THE importance of dose fractionation in influencing the magnitude of certain classes of cytogenic effects produced by sparsely ionizing radiations has been indicated repeatedly. Recently it has become apparent that the results of fractionation experiments are not always capable of a simple interpretation, as the response of cells can to some extent be affected by certain metabolic and physiological conditions. For example, WOLFF and his associates (WOLFF and LUIPPOLD 1958) and COHN (1958) have shown that metabolic conditions are important in determining the period of time during which chromosome breaks produced by one dose fraction can rejoin with those produced by a later fraction. Others (LANE 1952; HACQUE 1952; FETNER 1956; JAIN and MUJUMDER 1959) have indicated that one fraction can induce temporary physiological changes resulting in a protection against a later dose, though these results have been queried by some cytologists (for example, SAX and LUIPPOLD 1952). ELKIND and SUTTON (1959) state that in mammalian cells exposed to X rays, the kinetics of recovery after dose fractionation depend on the physiological state of the cells. Our earlier experiments (DAVIES and WALL 1960a) raised yet another difficulty in the interpretation of dose fractionation work. We demonstrated that splitting a dose into two or more fractions resulted in a reduction in the number of mutations produced, to a level below the base line expected on classical theory. A series of experiments to clarify the nature of this effect are reported here.

MATERIALS AND METHODS

Clonal material of *Trifolium repens* heterozygous for the leaf-marking alleles V^{bij} and v (BREWBAKER and CARNAHAN 1956) was used for all experiments. Six plants constituted one treatment sample. Details of methods of scoring somatic mutations at the V^{bij} locus, and experimental techniques have been described previously (DAVIES and WALL 1960a). Cobalt 60 gamma rays were used throughout. Plants were maintained in a controlled environment for 24 hours before the first fraction, between fractions and for 24 hours after the second fraction. After this period they were maintained in a greenhouse.

The absolute yield of mutations varies from one experiment to another. This is due to the fact that the number of mutations scored increases up to a given

sampling time after irradiation, then tends to fall off, whereas the number of leaves increases exponentially all the time, and total percentage mutant leaves will therefore depend on the environmental growing conditions (uncontrolled at present) and the stage at which scoring is terminated. The different experiments reported here were undertaken at various seasons and hence are not comparable, but within any given experiment valid comparisons may be made. No mutations occurred in control series. The oxygen used in the later experiments described was obtained from commercial cylinders (99.5 percent oxygen); the nitrogen used, contained 99.9 percent nitrogen and less than ten vpm of oxygen.

RESULTS

The effect of fractionation interval on yield of mutations: In this first experiment it was shown that dose fractionation led to a marked decrease in the yield of mutations, the reduction being most marked with fractionation intervals of two to eight hours. The effect of an acute dose of 1200 rads given in air in the dark, at a rate of 1050 rads per hour, was compared with that of two fractions of 600 rads given at the same dose rate, under similar conditions, and separated by various intervals of time ranging from two to 96 hours. Temperature during and between treatments was maintained at 25° ± 1°C, and the plants were maintained in the light between fractions. The percentages of mutations produced with the various treatments are given in Table 1. χ^2 tests of heterogeneity showed that there was no significant variation between the six replicate plants within any given treatment. Dose fractionation had no effect on leaf number but a marked one on the percentage mutant leaves. With a 2 to 8 hour interval there was a decrease to a value less than that expected from the additive effects of the two separate doses of 600 rads (cf. dose-response curve in DAVIES and WALL 1960a). Longer intervals between fractions led to an increase above this low level.

The interrelation of fractionation time and temperature: There was a greater reduction in mutation yield after dose-fractionation at low than at high temperatures. The maximum reduction was achieved after a longer interval at the lower temperature and no later recovery was observed (Table 2). The experiment

TABLE 1

Effect of fractionation interval on yield of mutations

Dose I in rads	Interval in hours	Dose II in rads	No. normal leaves	Percent mutant leaves
1200	0		5529	1.04
600	2	600	5710	0.63*
600	4	600	6547	0.67*
600	8	600	6571	0.66*
600	24	600	5301	0.88
600	48	600	6542	0.97
600	96	600	5428	0.80

* Values significantly different from single dose. P = 0.05 to 0.01.

which demonstrated this was similar to the previous one except that in one series the plants were maintained for 24 hours before irradiation, in the interval between irradiations, and for 24 hours after the second irradiation at 25°C and in the other series at 8°C. In the former the response was similar to that described previously though the recovery with longer intervals was not as marked. An analysis of variance of the two sets of results at 25°C showed no significant time interval × experiment interaction, indicating that both series responded in a similar manner.

In the 8°C series, the single dose treatment gave a higher yield of mutations; this was probably attributable to the higher oxygen concentration in the tissues at the lower temperature (DAVIES and WALL 1960b). With increasing time interval between fractions there was a continuous decrease in mutation frequency, and no recovery, at least within the time intervals used, as in the 25°C series. The maximum reduction was observed after 96 hours at 8°C, but after only two hours at 25°C.

Dependence of the fractionation response on the initial dose: Having demonstrated a significant reduction in mutation yield after certain dose fractionations, in this experiment the amount of the reduction, or "protection" and its possible dependence on the size of the initial dose was studied. A two-hour interval had proved an adequate time for the development of the "protective" effect at 25°C in earlier experiments, and this interval was chosen as the standard one here, though in two instances an eight-hour interval was also used (Table 3). Experimental conditions were similar to those in the previous 25°C series, and the dose was given in air in the dark at a rate of 2000 rads per hour. The numbers of mutations obtained with single doses of 100 to 2000 rads, and with a dose of 2000 rads preceded two hours earlier by a range of doses from 0 to 2000 rads was

TABLE 2

Effect of fractionation interval and temperature on yield of mutations

Temperature in °C	Dose I in rads	Interval in hours	Dose II in rads	No. normal leaves	Percent mutant leaves
25	1200	0	...	4022	2.38
	600	2	600	3996	1.65*†
	600	8	600	3668	1.58*
	600	24	600	4090	1.52*
	600	48	600	4411	1.76*
	600	96	600	4174	1.65*
8	1200	0	...	3923	2.92
	600	2	600	3984	2.23
	600	8	600	3968	2.27
	600	24	600	3971	1.63***
	600	48	600	4270	1.45***
	600	96	600	3873	1.30***

* $P = 0.05$ to 0.01.
*** $P < 0.001$.
† Values significantly different from single dose.

determined, and the results are presented in Table 3 and Figure 1. Again they show quite clearly that dose-fractionation results in a reduction of mutations recovered to a value below that of the additive effects of the two separate fractions —that is, an induced protection. An analysis of the difference between 2000 rads alone and 2000 rads preceded by 100 rads shows that the latter is significantly lower than the former—$x^2 = 8.63$, $P = 0.01$–0.001. With an eight, as opposed to

TABLE 3

Effect of initial dose on fractionation response

Dose I in rads	Interval in hours	Dose II in rads	No. normal leaves	Percent mutant leaves
...	..	100	3858	0.21
...	..	500	3387	0.59
...	..	1000	3618	1.84
...	..	1500	3399	3.57
...	..	2000	3325	4.86
100	2	2000	3334	3.45
100	8	2000	3503	3.71
500	2	2000	3094	3.73
1000	2	2000	3591	4.60
1500	2	2000	3297	5.77
2000	2	2000	2892	7.43
2000	8	2000	2765	6.56

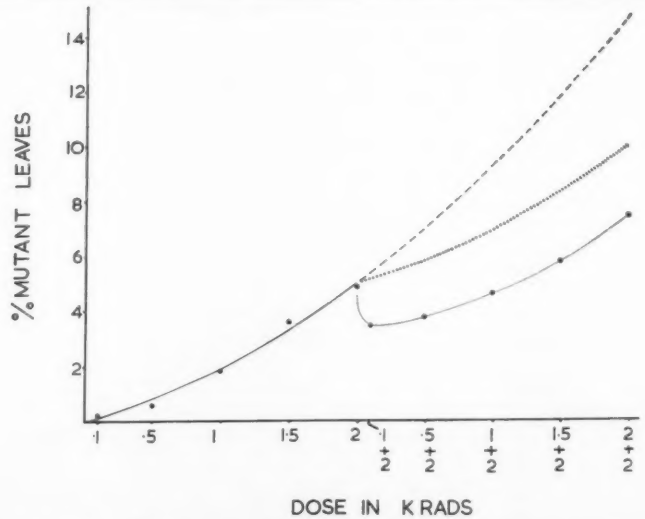

FIGURE 1.—Percentage mutant leaves produced by doses of 100 to 2000 rads and by similar doses followed two hours later by a dose of 2000 rads. — — — — extrapolated values for higher doses; ..... additive effects of two doses. Temperature 25°C.

a two-hour interval between fractions of 100 and 2000 rads, it appeared that the protective effect was being dissipated; when a higher initial fraction, 2000 rads, was used, the protective effect was still developing after eight hours (Table 3). Higher initial doses tended to induce a greater degree of protection than lower ones.

Statistical analyses of the dose response data were undertaken, involving fitting linear and quadratic functions to the percentage mutant leaves and the dose, in the ranges 0 to 2000 rads and 100 plus 2000 to 2000 plus 2000 rads. Both gave much better agreements with quadratic than with linear functions. For the acute series the relationship is yield $(y) = 9.925 \times 10^{-4} + 8.254 \times 10^{-6}x + 8.4943 \times 10^{-9}x^2$ where x is the dose, $x^2_{(3)} = 4.357$, P = 0.3 to 0.2; for the fractionated series the relationship is $y = 3.393 \times 10^{-2} + 3.276 \times 10^{-6}x + 8.446 \times 10^{-9}x^2$, $x^2_{(3)} = 0.02$, P = 0.99. The linear, but not the quadratic, coefficients of the two curves differed significantly. This is in direct contrast to most other dose-fractionation and chronic irradiation experiments.

Since the protective effect envisaged is almost certainly already developing while the larger doses are being administered, this will lead to flattening of the dose-response curve at higher doses. Information obtained from the curve regarding the proportion of one-hit and two-hit events will therefore not be wholly valid.

Low initial doses and short fractionation intervals: Following the results of the last experiment an investigation was undertaken of the effects of very low initial doses (12.5 to 100 rads) and of short fractionation intervals (5 to 120 mins.) on the development of the "protective effect". Again experimental conditions were identical with those in Experiment 2. 12.5 rads produced a marked reduction in the mutagenic effect of a subsequent main dose of 2000 rads (Table 4). An analysis of variance showed that pretreating with any dose from 12.5 to 100 rads prior to giving a dose of 2000 rads two hours later, resulted in a signifi-

TABLE 4

Effect of low initial doses and of short fractionation intervals on yield of mutations

Dose I in rads	Interval in mins	Dose II in rads	No. normal leaves	Percent mutant leaves
...	..	2000	4185	2.70
12.5	120	2000	4506	1.89*†
25	120	2000	4249	2.01*
50	120	2000	4508	1.64**
75	120	2000	4866	1.72**
100	5	2000	4458	2.13
100	30	2000	4385	2.43
100	60	2000	4492	2.11
100	120	2000	4475	1.92*

* P = 0.05 to 0.01.
** P = 0.01 to 0.001.
† Values significantly different from single dose.

cantly lower number of mutations than if 2000 rads had been given alone ($P = 0.01$ to 0.001). There were no significant differences between the five initial doses used. When the different fractionation intervals (five to 120 mins.) were compared, using a standard initial dose of 100 rads and a main dose of 2000 rads, there was no significant reduction in the mutation frequency until 120 minutes had elapsed between the doses, ($P = 0.02$ to 0.01) (Table 4), but the trend towards a lower value appeared even with a five-minute interval at this temperature (25°C).

Irradiations in air and in nitrogen: Since this reduction after fractionation could conceivably be related to an effect of the initial dose on oxidative metabolism and hence on intracellular oxygen tension, the influence of oxygen on the system was determined. Plants were placed in darkened chambers which were evacuated and flushed a few times with nitrogen or air prior to maintaining them in these respective atmospheres for a total time of 5½ hours with the 4000 rads and for 3½ hours with the 3000 rads series. Immediately postirradiation all plants were returned to normal aerobic conditions. The air:nitrogen ratio for the yield of mutants at each of two fixed doses was 2.3:1 (Table 5), a value in good agreement with others obtained for diverse types of radiobiological damage.

The effect of administering the initial fraction in oxygen or nitrogen: A possible link between metabolism and the development of the "protective effect" was indicated from the different types of response observed at 25°C and at 8°C in the first experiments, and further evidence of such a connection was now sought. Plants were maintained in nitrogen for two hours, then given 100 rads in nitrogen, maintained for a further 90 minutes in this gas, then equilibrated in air for 30 minutes before receiving the main dose of 2000 rads in air at a rate of 2000 rads per hour. Comparable treatments were given to another sample of plants, but oxygen was substituted for nitrogen. All were maintained in darkened chambers for the duration of the treatments. As controls some were given 100 rads in nitrogen or oxygen. Others had no initial dose of radiation. The final comparison involved allowing for the oxygen effect found in Experiment 4, and comparing an initial dose of 100 rads in oxygen with 230 rads in nitrogen. All results and analyses are given in Table 6, and from these it is apparent that the marked "protective effect" observed in all previous experiments developed only if the initial fraction was administered in air. Transferring the plants immediately after irradiation in nitrogen to air had no effect (Table 6).

TABLE 5

Effect of irradiating in air or nitrogen

Dose in rads	Gas during irradiation	No. normal leaves	Percent mutant leaves	Air/N_2 ratio
4000	Nitrogen	3876	1.75	2.33
4000	Air	3531	4.07	
3000	Nitrogen	3679	1.37	2.31
3000	Air	3452	3.17	

TABLE 6

Effect of administering the initial fraction in oxygen or nitrogen

Treatment	Dose I in rads	Gas during dose I	Gas post-dose I	Dose II in rads	No. normal leaves	Percent mutant leaves
1	100	O_2	O_2	2000	4461	1.42
2	100	N_2	N_2	2000	4665	2.08
3	..	..	O_2	2000	5038	2.14
4	..	..	N_2	2000	5287	1.89
5	100	O_2	O_2	....	5137	0.19
6	100	N_2	N_2	....	5131	0.14
7	230	N_2	O_2	2000	4403	2.18
8	230	N_2	N_2	2000	4329	2.17

Comparison of 1 *vs.* 2. χ^2 5.247 P = 0.05 to 0.01.
Comparison of 1 *vs.* 3. χ^2 7.820 P = 0.01 to 0.001.
Comparison of 2 *vs.* 4. χ^2 0.440 P = 0.7 to 0.5.
Comparison of 1 *vs.* 7. χ^2 7.350 P = 0.01 to 0.001

DISCUSSION

In all experiments it was demonstrated that dose-fractionation had a pronounced effect on the number of mutations produced but none on leaf production, so that the effects observed were not attributable to gross changes in development or growth rates. The following specific points were established. (1) Certain dose fractionations led to a reduction in the number of mutations recovered to a value below the base line of the additive effects of the two fractions. (2) The development of this "protective" effect was dependent on time, being at a maximum at 2 to 8 hours at 25°C, on temperature, taking longer to develop at 8°C than at 25°C, and on dose, being greater with higher initial doses. (3) With high temperatures the effect diminished after long intervals, but persisted at lower temperatures. (4) Doses as low as 12.5 rads induced "protection," and the phenomenon developed within a very short period postirradiation. (5) "Protection" occurred only if the initial fraction was given in the presence of oxygen.

In Figure 2 the effect of dose fractionation is most clearly demonstrated. It shows how the number of mutant leaves produced by a dose of 2000 rads at 25°C, varies according to the initial dose given two hours earlier.

Possible explanations of the induced "protection" can now be considered. The first alternative is that the initial dose gave rise to a lowered oxygen tension in the tissues, the later fraction then acting on a wholly or partially anoxic system. The oxygen factor of 2.3 could account in theory for the observed protection, but the hypothesis is unacceptable. With the very long intervals between fractions used in the first experiments, sufficient oxygen could diffuse into the tissues to remedy any general induced anoxia. Again the absence of any effect when 230 rads were given in nitrogen is difficult to understand on this basis. It is of course conceivable that oxidative metabolism was affected. The effect of 100 rads on the respiratory gaseous exchange of the irradiated tissues was measured in a Warburg apparatus,

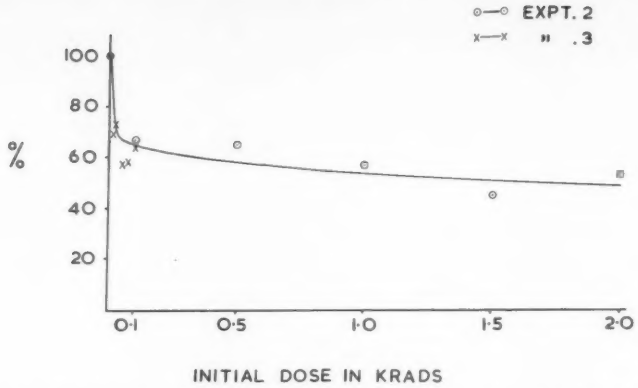

FIGURE 2.—Number of mutants produced by 2000 rads, following various initial radiation doses. Values expressed as a percentage of the number of mutants produced by 2000 rads without initial radiation treatment. (Fractionation period two hours, temperature 25°C).

and the respiratory quotient checked at ten-minute intervals for 150 minutes post-irradiation, but no change was observed, indicating an unaltered oxidative metabolism. This over-all negative result need not of course necessarily apply to all sensitive sites within the cell. No effect of such low doses on oxygen metabolism has been reported elsewhere.

Before discussing an alternative explanation, it is necessary to consider briefly the possible nature of the mutational events—whether they are due to chromosomal aberrations, to true point mutations at the nucleotide level, or to a mixture of both. If they are aberrations then they could be minute deficiencies or duplications, as these are types which could survive the considerable number of cell divisions intervening between the initiation and recognition of the mutational events. At least some must be due to aberrations as two-hit events were recovered. In Experiment 2, it was shown that the one-hit component was changed after fractionation, but the two-hit remained unaffected. With a two-hour interval between fractions and assuming a rejoining time of less than two hours, then the proportion of two-hit events produced by a dose of 2000 rads and by doses of, for example, 2000 plus 2000 rads, should on classical theory be similar. However, if the one-hit chromosome aberration component is affected by fractionation, then by definition the two-hit events should also be affected, but this did not occur. It is possible, therefore, that there is another one-hit component—that concerned with point mutations and capable of being modified or "protected."

The alternative explanation of the observed protection can now be considered. This is related to mitotic delay and will be discussed initially in relation to chromosomal aberrations. REVELL (1959) has shown that various stages of interphase differ slightly in sensitivity. Cells of *Vicia faba* root tips, two to six hours away from metaphase, in the previous interphase, were about one-and-a-half

times as sensitive as those ten hours away. If the initial fractions of radiation caused mitotic delay, then cells might be prevented from entering more sensitive stages by the time the main dose was given. However, it is doubtful whether doses as low as those used in our experiments, 12.5 rads, and administered in G_2 (post-synthetic period) could induce much delay (NEARY, EVANS, and TONKINSON 1959). It is of course possible that chromosome sensitivity alters after an initial fraction of radiation.

If mitotic delay is considered in relation to point mutation, then it is conceivable that radiation may inhibit certain processes which normally result in the conversion of premutational damage into mutation (compare DOUDNEY and HAAS 1958; WITKIN 1956). However, any such inhibition must also occur after an acute treatment, and to explain the induced protective effects observed in our experiments after pretreatment with low doses, it would have to be assumed that the conversion into mutation normally occurs almost instantaneously after exposure to an acute dose.

Another possible explanation of the induced protection again involves mitotic delay. This time it has to be considered in relation to the presence of a sensitive stage to point mutation induction in interphase. If we accept that a proportion of the observed mutations are point mutations, we can consider their production in relation to deoxyribonucleic acid (DNA) synthesis. Let us assume that the first fraction retards DNA precursor uptake (for evidence of accumulation of nucleotides in irradiated cells, see MANDEL and CHAMBON 1959), and further assume that some forms of radiation-induced mutations can be attributed to errors in nucleotide organization or the ordering of the base sequences. Having given the first low dose, the main dose can then produce very few such errors, if little or no nucleotide organization is occurring at that particular time. Errors of the type envisaged are thought to be the cause of base-analogue and formaldehyde-induced mutations (FREESE 1959; ALDERSON 1960). A link between DNA synthesis and mutation induction is also indicated in other work (GLASS and NOVICK 1959). Numerous workers have shown that DNA synthesis is depressed after irradiation, owing to mitotic delay and changes in the cell population, and to an effect on the biosynthesis of DNA itself. Certainly the system is one of the most sensitive known. CREASEY and STOCKEN (1958) showed that nuclear phosphorylation in rat lymph nodes and bone marrow is 100 percent inhibited three minutes after exposure to 50 to 100r. Again reduction of precursor uptake into DNA is detectable in rat thymus after 25r (ORD and STOCKEN 1956). Furthermore, they (ORD and STOCKEN 1958) have shown that after total body exposure of rats to X rays there is a reduction in the incorporation of P^{32} into the thymus DNA two hours later. The reduction is marked and linear from 25 to 200r, but at higher doses there is only a slight further reduction. There is therefore the possibility that the protective effect of very low doses, and the dose-protection curve for mutation (Figure 2) may involve or be compared with, the effects of radiation on DNA precursor uptake. Similarly, it is worth examining our other observations in this context. The rapid drop in mutation frequency with short fractionation intervals

followed by an increase in the frequency at longer ones is analogous to the observations of CATTANEO, QUASTLER, and SHERMAN (1960), who have observed a reduction and a later increase in the amount of tritiated thymidine incorporated into DNA after X irradiation of mouse hair follicles. In their data, as in ours, there is the conflicting observation of a reduction within a very short interval of exposure. This implies an effect on S (synthetic period)—a stage previously considered by most but not all workers (see PAINTER and ROBERTSON 1959) to be resistant. Again the longer lasting protection observed at 8°C in the present experiments, the greater effect at the low temperature and with high doses, can now be interpreted in terms of differential rates of recovery of some synthetic systems.

The role of oxygen in the production of the protective effect is not obvious, but it may have to be present in certain vital sites before they become radiosensitive. ORD and STOCKEN (1958) have suggested that the radiosensitivity of nuclei and mitochondria and the relative resistance of, for example, microsomes, can be correlated with the participation of oxygen in a bound form in some of the former's reactions. PAUL (in press and personal communication) has obtained evidence that a depression of tritiated thymidine incorporation into DNA by radiation occurred only if the medium was equilibrated with air or oxygen during irradiation. These many similarities strengthen the possibility of a link between the observed radiation induced mutations and DNA synthesis.

If the theory of radiation induced errors in DNA organization is accepted, then recognizable mutational changes of this sort can occur only after the completion of at least two subsequent replications, since TAYLOR, WOODS, and HUGHES (1957) model of chromosomal duplication has placed DNA replication at the subchromatid level. Furthermore, no dose rate or dose-fractionation effect on mutation induction would be expected, and indeed none has been observed, with stages not actively synthesizing DNA, for example, mature sperm. But dose-rate effects would be expected and are found with other stages (RUSSELL, RUSSELL, and KELLY 1958). After irradiation of immature stages some mosaic progeny might be expected as a result of the segregation of mutant and nonmutant strands. Our results imply that some particular stage of interphase is particularly sensitive to the production of the type of mutational event we are concerned with here. Under chronic irradiation conditions it is possible that induced mitotic delay could be much greater for a mutation-insensitive than a mutation-sensitive stage, and hence in a given population of cells, many fewer will be at the mutation-sensitive stage at any given time. The data of WIMBER (1960) exemplify this differential response. Plants equilibrated under chronic irradiation conditions had G_1 (presynthetic period) increased by a factor of 13.75 whereas S was increased by a factor of only 1.57.

The implications of these results and of the possible presence of a sensitive stage are being analyzed at present in further experiments.

SUMMARY

1. Dose fractionation led to a reduction in the number of mutations recovered to a value below the expected base line of the additive effects of the two fractions.

2. The development of this protective effect was dependent on time, being at a maximum at 2 to 8 hours at 25°C; on temperature, taking longer to develop at 8°C than at 25°C; and on dose, being greater at higher doses.

3. With high temperatures the effect diminished after long intervals, but persisted at lower temperatures.

4. Doses as low as 12.5 rads induced protection, and the phenomenon developed within a very short period postirradiation.

5. Protection occurred only if the initial fraction was given in the presence of oxygen.

6. The protective effect observed could possibly be attributed to mitotic delay and the presence of a sensitive stage in interphase for the induction of some point mutations.

7. Attempts have been made to correlate the observations with the known effects of radiation on DNA synthesis.

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CHROMOSOMAL POLYMORPHISM IN DROSOPHILA RUBIDA MATHER

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THE population geneticist is essentially interested in the variation of gene frequencies in natural populations. Within chromosomal inversions blocks of genes are contained and in many species of Drosophila due to the presence of easily analysable giant chromosomes, the behavior of these blocks of genes can be easily studied in natural populations. Thus, of recent years chromosomal inversion polymorphism in the genus Drosophila has been extensively studied (see review by DA CUNHA 1955 and discussion by GOLDSCHMIDT 1958), the most extensive work having been done on the temperate species *D. pseudoobscura* Frolowa from northwestern America, *D. robusta* Sturtevant from eastern America and *D. subobscura* Collin from Europe and the tropical species *D. willistoni* Sturtevant from South America. From this work it has been suggested that the genetical significance of inversions is to maintain coadapted gene sequences by the elimination of chromatids produced by crossing over within the limits of heterozygous inversions.

PATTERSON and STONE (1952) list 17 species in the *immigrans* species group, the majority being from the Australian and Oriental geographical regions. In spite of the giant chromosomes of those members of the group which have been studied being very suitable for detailed analysis, only *D. immigrans* has been examined for polymorphism. A number of inversions have been detected by FREIRE-MAIA, ZANARDINI and FREIRE-MAIA (1953) and BRNCIC (1955) in South America, GRUBER (1958) in Israel and TOYOFUKU (1957, 1958a,b) in Japan.

In 1958 Drosophila collections were made at Cairns in northeastern Australia and a new species of the *immigrans* species group discovered. This was subsequently described as *D. rubida* (MATHER 1960) and shown, like *D. immigrans*, to have excellent salivary chromosomes although the Cairns population appears to be free of inversions.

In 1959 Drosophila collections were made in Papua-New Guinea at Port Moresby, Goroka and Lae (New Guinea), Kavieng (New Ireland) and Rabaul (New Britain), and the species was shown to be widespread (MATHER 1959). It was also established by qualitatively analysing a number of mass cultures that it is rich in chromosomal inversion polymorphism. For these reasons it was decided to study *D. rubida* more fully with a view to obtaining data on the polymorphism pattern of a polymorphic species from tropical Australasia.

[1] Aided by a grant from the University of Queensland Research Fund.

MATERIALS AND METHODS

The flies used in this study were collected from fermenting banana baits exposed in rain forests at Cairns, Port Moresby, Goroka, Lae and Bulolo (New Guinea) and Rabaul (New Britain) (Figure 1). Thus the sampling stations are spread over a considerable geographical area as Rabaul is nearly 1000 miles from Cairns and whereas Cairns, Port Moresby, Lae and Rabaul are at sea level, Goroka and Bulolo are at heights of 5000 and 2500 ft., respectively.

In order to extract the maximum amount of information from the samples collected, three complementary methods of analysis were made (DOBZHANSKY and EPLING 1944; STALKER 1960).

(1) Wild-caught males were analysed by mating them singly to virgin females from a standard strain (Cairns, May 1958) which has been shown to be inversion free. Seven larvae of which at least one was a female were scored.

(2) Wild-caught females probably inseminated but kept until no fertile eggs were laid (i.e. despermed) were analysed by mating them singly to males from the standard strain and scoring seven female larvae.

(3) The male progeny of females caught in the wild were tested as wild males (see (1) above). Experimental justification for this procedure is given by STALKER

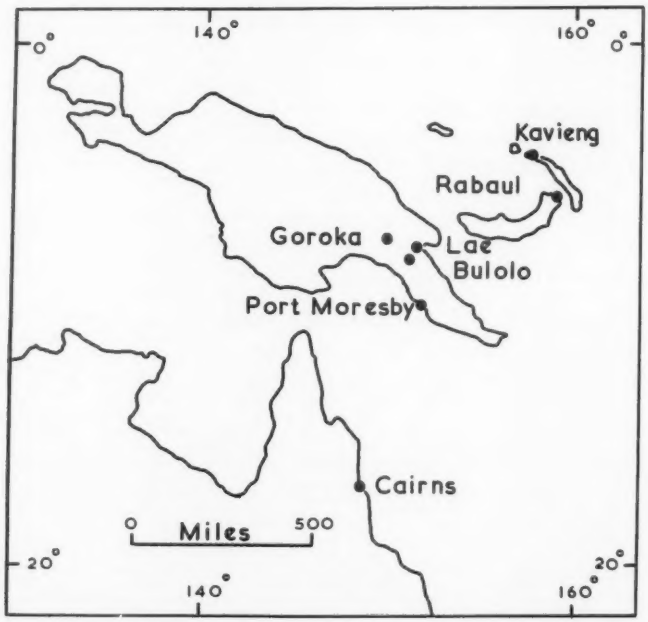

FIGURE 1.—Sampling stations in Northern Australia and the Territory of Papua and New Guinea.

(1960) who found that a comparison of wild-caught males, and the F_1 sons of wild-caught females captured at the same time in a large population of *D. paramelanica* in Iowa showed no difference in inversion frequencies between the two classes. Presumably F_1 females could be used in the same way but the validity of this has not been tested.

It can be shown that by using seven larvae from each mating that there is over a 98 percent probability of testing *both* haploid chromosome sets from each individual (DOBZHANSKY and EPLING 1944). Further, because in each method a wild individual is mated to a standard gene order individual, the gene order of the chromosome under test can always easily be determined for males, but due to crossing over in females, the linkage relationships are sometimes difficult to determine.

Wild-caught females may be used, but here, to give a comparable probability of testing both haploid chromosome sets from each individual, eight instead of seven larvae must be used (DOBZHANSKY and EPLING 1944). Also the possibility of multiple insemination introduces uncertainty. For estimating the frequency of inversions in populations, single larvae from each female inseminated in the wild have been used by a number of workers.

In testing female flies, female larvae must be used to ensure testing both X chromosomes. In testing male flies at least one female larva must be used to be certain of testing the X.

When analysing a complex inversion, a sketch was made, double and single thickness regions being particularly noted, both the standard and nonstandard halves traced out and each paired or homologous section lettered at both ends. Finally the sequence of letters in each half giant chromosome was noted and the minimum number of simple inversions deduced (DOBZHANSKY and EPLING 1944).

The flies were grown on standard corn meal medium at 25° ± 1.0°C, salivary gland squashes made in 45 percent acetic acid after pretreatment with N HCl and staining in aceto-orcein. The squashes were scored as paraffin sealed temporary mounts. All photographs were made on 35 mm Panatomic X film.

Chromosomes: D. rubida has a metaphase plate of two pairs of V's and two pairs of rods and a giant chromosome complement of four long arms and one dot. Thus, in evolving from the supposed primitive condition of five pairs of rods and a pair of dots the most likely explanation in the light of the cytology of the closely related *D. pararubida* (MATHER 1961) seems to be that two pairs of rods fused but in one pair a subsequent pericentric inversion has produced a pair of long rods. Further, heterochromatin has been added to the original dots to produce a pair of rods and to the remaining original rods to produce another pair of V's.

The giant salivary chromosomes show four long arms and one short and the arms can be readily identified by the banding of the free ends. The same designation has been used as that given for *D. immigrans* (FREIRE-MAIA *et al.* 1953), *viz.* I for the X chromosome, IIL and IIR for the arbitrary left and right arms

of one pair of metaphase V's, III for the long metaphase rod and IV for the small arm representing the euchromatic part of the largely heterochromatic metaphase rods. The X chromosome is further specified by being more lightly stained than the autosomes in preparations from male larvae. The third chromosome is nearly twice as long as IIL or IIR. There is no distinct chromocentre and IIL often appears to be continuous with IIR whereas I and III tend to separate at their centromere ends and from IIL and IIR. Chromosome IV is usually attached to the centromere end of one of the other chromosomes.

RESULTS

A giant chromosome photographic map (Figure 2) has been prepared from the standard inversion-free Cairns strain by systematically photographing clear preparations under oil immersion and reconstructing the chromosomes from the prints produced. Each chromosome arm has been broken up into regions working from the free end towards the centromere. The regions begin with easily identifiable bands spaced at such distances that within each region approximately ten bands were distinguishable and indicated by dots. Thus a breakage point designated IIR 2.3 means that the break occurs immediately to the right of band 3 in region 2 of the right arm of chromosome II. The use of a photographic giant chromosome map instead of the more conventional camera lucida drawn map was first suggested by CARSON (1958) and has been adopted here in the belief that this type of map is of greater use to subsequent workers in that a particular break point can be more definitely designated. Photographs of the simple inversions detected are given in Figure 3 and the complex inversions IIR D and E, together with interpretations, are given in Figure 4 with the aim of giving concrete evidence of the position of the postulated break points.

Chromosome I, the X chromosome, is free of inversions. IIL has one simple inversion A(7.1–10.5) towards the proximal end involving 29 percent of the giant chromosome.

The distal end of IIR has a subterminal simple inversion A(1.2–3.3) involving 11 percent of the giant chromosome. This is followed by a further simple inversion B(3.3–5.5) apparently with the same distal break point and involving 17 percent of the chromosome. Finally, simple C(7.1–9.4) and complexes D(7.1–14.1) and E(7.1–16.1) each have the same distal break point but different proximal break points. Complex D contains seven included inversions with the following break points 7.1–14.1, 7.1–9.4, 9.4–14.1, 8.5–9.4, 9.4–10.7, 10.7–14.1, 12.2–14.1. Thus simple C is included in complex D. Complex E contains six included inversions with the following break points 7.1–16.3, 9.4–16.3, 9.4–10.7, 10.7–14.1, 14.1–16.3, 12.2–14.1. Thus three of these inversions, *viz.* 9.4–10.7, 10.7–14.1, and 12.2–14.1 also occur in complex D. Simple C involves 14 percent, complex D 40 percent, and complex E 51 percent of the chromosome.

Chromosome III has four simple inversions, A(2.0–3.7), B(6.4–8.0), C(18.6–21.4), D(24.0–26.0) spread out along its length and involving 8, 7, 9.5 and eight percent of the chromosome.

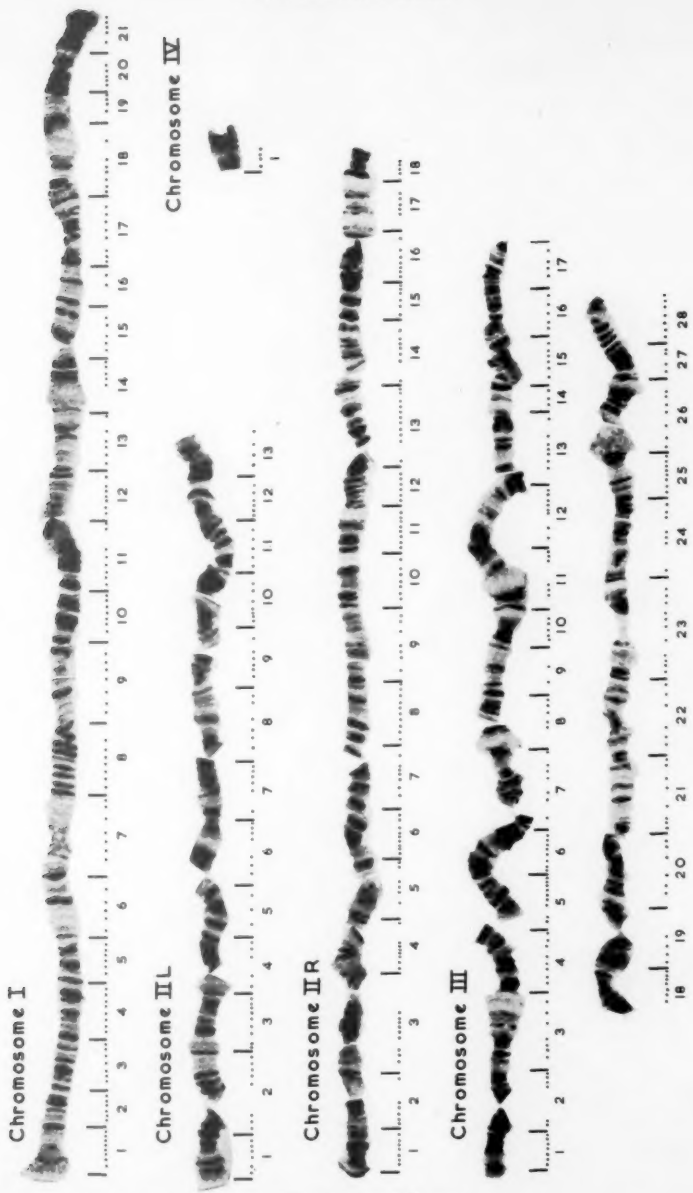

FIGURE 2.—*D. rubida* giant chromosome photographic map.

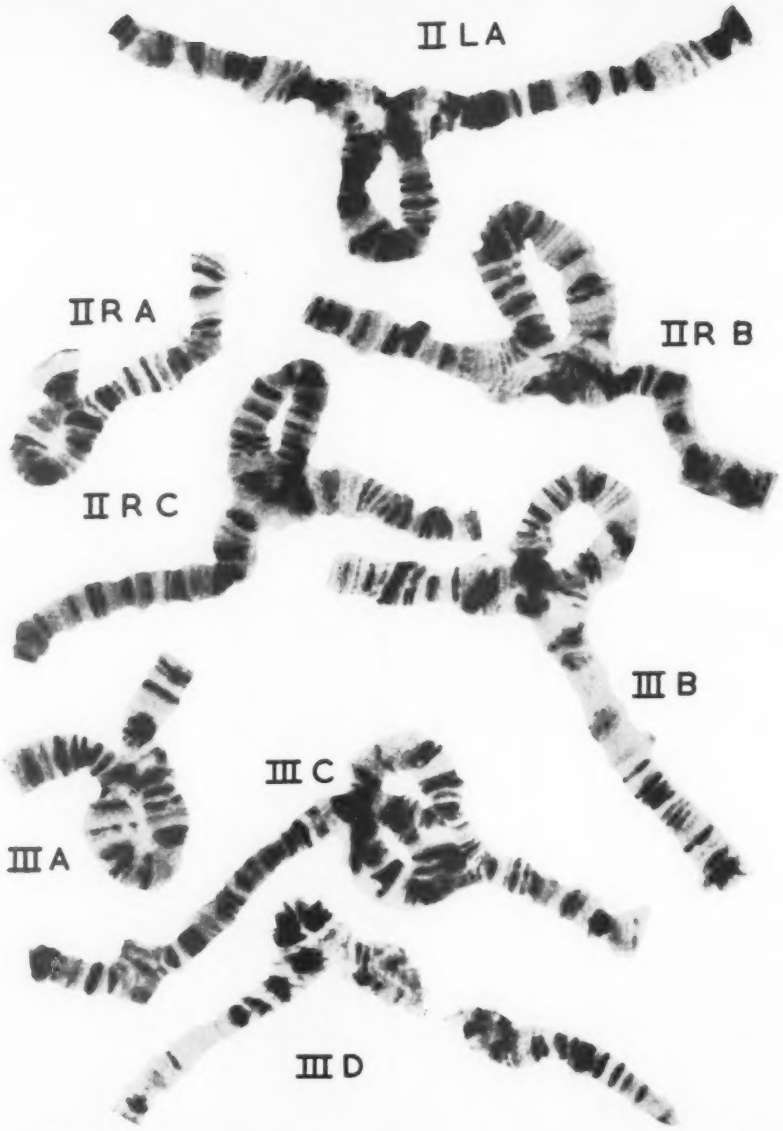

FIGURE 3.—Heterozygous simple inversions.

Thus in all there are 17 inversions so far detected in *D. rubida*.

The frequencies of the gene sequences present are given in Tables 1, 2 and 3. Population samples of a size adequate for statistical analysis were obtained only at Port Moresby and Bulolo. The presence (+) or absence (−) of the respective inversions at other localities is also shown, as well as the number of individuals available and the number of chromosomes tested. The *D. rubida* population at Cairns was free of inversions and was used as the standard sequence. The most prevalent gene sequence for all chromosomes at Moresby (near sea level) is the

FIGURE 4.—Heterozygous complex inversions. In IIR E a b and a′ b′ are homologous regions. The standard sequence is shown in black.

TABLE 1

Gene order frequency of chromosome IIL

	Moresby percent	Goroka	Rabaul	Bulolo percent	Lae	χ^2	P
Gene order							
+	98	+	−	84	+	...	..
A	2	+	+	16	−	4.40	<.05
Chromosomes tested	50	2	14	50	4	...	..
Wild males	16	1	7	19	..	...	..
Despermed females	4	..	..	6	2	...	..
F_1 males	5	..	..	..	..	...	..

TABLE 2

Gene order frequency of chromosome IIR

	Moresby percent	Goroka	Rabaul	Bulolo percent	Lae	χ^2	P
Gene order							
+++++	54	—	—	—	—	...	..
++C++	10	—	—	—	—	...	..
+B+D+	22	+	—	32	+	0.81	>.05
+++D+	10	—	—	30	—	5.06	<.05
AB+D+	2	—	—	4	—	0.00	..
A++D+	2	+	—	34	—	15.24	<.01
++++E	—	—	+	—	—	...	..
Chromosomes tested	50	2	14	50	4	...	..
Wild males	16	1	7	19	..	...	..
Despermed females	4	..	..	6	2	...	..
F_1 males	5	..	..	..	..	...	..

TABLE 3

Gene order frequency of chromosome III

	Moresby percent	Goroka	Rabaul	Bulolo percent	Lae	χ^2	P
Gene order							
++++	74	—	—	42	—	9.24	<.05
++C+	20	—	—	4	—	4.64	<.05
+++D	2	—	+	26	—	10.05	<.01
AB++	2	—	—	—	—	...	..
++CD	2	+	—	28	—	11.29	<.01
A++D	—	—	+	—	—	...	..
+BCD	—	—	—	—	+	...	..
Chromosomes tested	50	2	14	50	4	...	..
Wild males	16	1	7	19	..	...	..
Despermed females	4	..	..	6	2	...	..
F_1 males	5	..	..	..	..	...	..

standard sequence, while there is a marked increase in inversion frequency at Bulolo (2500 ft.).

Comparing Port Moresby and Bulolo from which reasonable samples are available, it can be seen from Table 1 that inversion A is at a significantly greater frequency at Bulolo than Moresby. From Table 2 it is seen that the standard gene order and ++ C ++ represented at Port Moresby are lacking at Bulolo, complex inversion D appears to be fixed at Bulolo and complex E occurs only at Rabaul. +++ D + and A ++ D + gene orders present at Port Moresby are at a significantly higher frequency at Bulolo. Likewise in chromosome III (Table 3) there are significant differences in the frequencies of gene orders at Port Moresby and Bulolo.

The inversions appear to be concentrated on chromosome IIR and to a lesser extent on III whereas IIL has only one inversion and chromosome I none. Assuming that the Hardy Weinberg law is applicable, as indicated in Table 4, the

TABLE 4

Non random association of inversions IIRB and IIRD—Bulolo

Chromosome	Observed	Expected
++	31	24.42
BD	13	4.42
+D	5	12.58
B+	1	8.58
Total	50	50
	χ^2 for 1 d.f. = 4.05 ∴, P < .05	

expected numbers of the four sequences ++, BD, +D and B+ in chromosome IIR at Bulolo can be calculated from the inversion frequencies in the population. When this is done and compared with the observed sequences significant non random association is shown.

It will be noted that heterozygosity in chromosome IIR is partially maintained by complexes D and E but with the exception of C the simple components of D and E are absent from the populations. Even C with a frequency of ten percent at Moresby is rare.

The mean inversion heterozygosity at Port Moresby is 2.6 ± 0.6 S.E. (range 0–9) and at Bulolo is 2.3 ± 0.3 S E. (range 0–5) which does not differ significantly (P > .05, t = 0.4). Applying the index of crossing over devised by CARSON (1955) Port Moresby has one of 94 ± 1.4 S.E. and Bulolo 92 ± 1.2 S.E. which once again are not significantly different (P > .05, t = 1.1). This is sharply contrasted with the situation at Cairns where no inversions have been detected in six mass cultures collected in May 1958.

DISCUSSION

The 17 inversions so far detected in *D. rubida* allows it to be classified as one of the more polymorphic Drosophila species. Of the 114 Drosophila species examined for inversion polymorphism, 17 have 15 or more inversions and the mean is 6.0 ± 8.4 S.E. (LEVITAN 1958). The only other members of the immigrans group that have been examined for inversion polymorphism are *D. immigrans* with four simple and four complex inversions, *D. pararubida* (MATHER 1961) and *D. setifemur* (MATHER, unpublished) with none. In *D. immigrans* from South America (FREIRE-MAIA *et al.* 1953; BRNCIC 1955) one inversion occurs in in the middle part of IIL and the two others at the subterminal distal part of IIR and in the proximal part of IIR. In *D. immigrans* from Israel only the IIL inversion has been recorded. In *D. immigrans* from Japan (TOYOFUKU 1957, 1958a,b) two simple and four complex inversions have been detected. One at the distal end of IIR appears to be the same as the most distal IIR simple inversion from South America and Israel. Also three complex inversions appear to occur at the distal end of IIR, a simple inversion near the centre of III and a complex inver-

sion also near the centre of III. Thus, although the components of the complex inversions are not indicated, the inversion polymorphism in *D. immigrans* from Japan bears a closer resemblance to that of *D. rubida* than does that from South America or Israel. However, because *D. rubida* and *D. immigrans* will not hybridize (MATHER 1960) the relationships of the *D. immigrans* to the *D. rubida* inversions remain uncertain.

The considerable geographical variation in frequency and in some cases replacement of gene order in *D. rubida* resembles the situation in *D. pseudoobscura*, *D. robusta*, *D. subobscura* and *D. willistoni*.

In *D. rubida* the inversions appear to be concentrated in certain chromosomes resembling more the situation in *D. pseudoobscura* than that in *D. willistoni*, *D. robusta* and *D. subobscura* where the inversions are more or less evenly distributed between the chromosomes. LEVITAN (1958) has extensively studied non random association of inversions in *D. robusta* and drawn attention to cases in other species. Here in *D. rubida* there is clear-cut evidence of non random association between IIR B and IIR D presumably because of the higher adaptive value of the favoured linkages.

DOBZHANSKY and STURTEVANT (1938) classified multiple inversions occurring in the same chromosome into three classes (*viz.* overlapping, included and independent). They also pointed out that only with overlapping inversions is it possible to trace phylogeny and thus the phylogenies of the inversions in complexes IIR D and IIR E, being of the included type cannot be traced. WALLACE (1953) using data from *D. pseudoobscura* and *D. robusta* has pointed out that in species having overlapping inversion complexes where there are "triads" of three inversions, there can be a breakdown of coadapted gene sequences through crossing over unless one member of the triad is in low frequency or absent. This reasoning cannot be applied directly to included inversions and in any case there are here no "triads" of included inversions. However, where a given region of a chromosome is prevented from crossing over by a heterozygous inversion and if the inversion is large, multiple crossovers can lead to the nonelimination of chromosomes produced by crossing over, but nevertheless, the coadapted gene sequences can be broken up. This possibility is reduced by multiple inversions in a complex and may well explain the apparent adaptive superiority of the complexes over the hypothetical less complex or simple inversions which are the components of the recorded complexes.

LEVITAN (1958) has reviewed the literature on inversions in Drosophila and shown the occurrence of complexes to be very widespread. Thus the occurrence of complexes in *D. rubida* is not unexpected. However, as LEVITAN points out, included complexes rarely involve more than three inversions and in IIR D of *D. rubida* there are seven and in IIR E there are six.

LEVITAN also points out that theoretically the members of an included complex can be assorted by crossovers. However, this is undoubtedly very rare due to the fact that for this to occur the crossovers must be coincidentally outside the limits of the shortest inversion but within the limits of the longest inversion.

DA CUNHA and DOBZHANSKY (1954) put forward the hypothesis that inversion heterozygosity falls off towards the periphery of the range of a species. It has been noted above that inversion heterozygosity appears to be absent at Cairns and this is possibly near the periphery of the range. However, there is no significant difference in heterozygosity between Moresby and Bulolo. Further data will undoubtedly throw light on this aspect of the over-all problem.

SUMMARY

1. A photographic giant chromosome map of *D. rubida* is presented and the positions of 17 inversions from a sample of flies taken from five stations in Papua and New Guinea are recorded.

2. There is considerable geographical variation and in some cases replacement of the gene orders present.

3. There is marked concentration of the inversions and a case of non random association of inversions in chromosome IIR.

4. Both simple inversions and included complexes are utilized in maintaining heterozygosity.

5. An explanation of the very high adaptive values of two included complexes over less involved complexes is given in terms of coadaptation.

6. Inversion heterozygosity frequency varies sharply between certain geographical regions.

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TEMPERATURE EFFECTS ON LETHAL MUTATION RATES OF HABROBRACON OÖCYTES X-IRRADIATED IN FIRST MEIOTIC METAPHASE[1]

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A BRIEF note was published in 1940 (WHITING) concerning the influence of temperature on X-ray sensitivity of different meiotic stages in the Habrobracon oöcyte. Unmated females were kept at 0°C, 13°C, 25°C, and 35°C for one hour before and one hour after irradiation. During a one half minute of exposure to 212r, they were at room temperature. For oöcytes in first meiotic metaphase (metaphase I), mortality was lowest at 0°C, highest at 25°C and intermediate at 35°C. The consistent and extensive radiation data on metaphase I obtained since 1940 made it seem valuable to redesign and to repeat the experiment.

When Habrobracon females are X-irradiated, responses of their oöcytes in prophase I can be distinguished from those in metaphase I (WHITING 1945). The latter were used in the experiments reported in this paper. LD 50 for the stage is about 400r. The nature of the dose-action curve as well as the absence of any significant differences in response of this curve to different experimental conditions (for example, 124 kv, 50 mev by HEIDENTHAL, CLARK and GOWEN 1955; 100r/min X-rays, 200r/sec cathode rays by HEIDENTHAL 1960; 6.6r/min, 800r/min by LACHANCE 1960; and fractionation by WHITING 1945) furnish evidence for one-hit X-ray-induced changes. Tetrad conditions at the time of irradiation and cytological observations on succeeding stages also make the one-hit explanation a plausible one.

Viable eggs laid by unmated females develop into haploid males. Those in which dominant or recessive lethal mutations acting on the embryo are induced (as happens in the majority of eggs) fail to hatch.

MATERIAL AND METHODS

Virgin females were X-irradiated and bred unmated. Response of oöcytes irradiated in first meiotic metaphase was measured by egg hatchability.

[1] This investigation was supported by a research grant PHSC-741(C4) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, and a grant from the Phi Beta Psi Sorority. Able technical assistance was given by Miss Ruth Starrells.

[2] Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

For preirradiation low temperature experiments, well-fed females were kept without food for 16 hours, the first eight at 30°C followed by eight in the freezing compartment of a refrigerator (−3°C to −5°C). For low temperature during irradiation, females were put into chilled gelatin capsules which were placed in a small plastic dish submerged in ice and salt water. For postirradiation low temperature experiments, the females immediately after irradiation were kept for 40 minutes in the freezing compartment. For high temperature, 30°C was substituted for the −3°C to −5°C treatment. Both groups were irradiated at the same time, the higher temperature capsules being placed above the lower or *vice versa*. A dual-tube self-rectifying unit with a simultaneous cross-firing technique was used. Secondary voltage was 182 kv; tube current on each tube, 25 mA; and rate, 722 rpm. Doses were 1263r and 1444r.

Hatchability of eggs from nonirradiated females kept at −5°C for 12 hours was 98.1 ± 0.93 percent, for those kept at 30°C, 97.4 ± 1.04 percent.

The use of low temperature is indicated by −, of high by +. The conditions before, during, or after irradiation are designated by using the symbols sequentially. Thus, in − − − experiments, low temperature was used throughout; in + − + experiments, during irradiation only.

RESULTS

Results are presented in Table 1. The difference in hatchability between total − − − and + + + in experiments at 1263r was 11.07 ± 1.77 percent; at 1444r, it was 8.95 ± 2.33 percent. Arranged in the order of decreasing survival at 1263r, the results were − − −, − + +, + − −, − − +, + + +, + − +; for 1444r, − − −, + + −, + + +, + − +. It is apparent that, with reduction of low tem-

TABLE 1

Hatchability of unfertilized eggs X-irradiated in first meiotic metaphase. The − sign represents −3° to −5°C; + is 26° to 30°C. First − or + in a given series refers to temperature treatment before irradiation; second, during; and last, after

Dose (r)	Temperature	Hatchability (Viable/total) (Percent viable)	Temperature	Hatchability (Viable/total) (Percent viable)	P Approx. significance level of difference
1263	− − −	89/441 = 20.18	+ + +	43/351 = 12.25	0.001
	+ − +	12/116 = 10.34	+ + +	12/124 = 9.67	0.86
	− + +	41/247 = 16.59	+ + +	28/320 = 8.75	<0.01
	+ − −	47/297 = 15.82	+ + +	31/296 = 10.47	0.05
	− − −	65/302 = 20.73	− − +	43/285 = 15.09	0.07
	− − −	154/743 = 21.52*	+ + +	114/1091 = 10.45*	<0.001
1444	− − −	33/246 = 13.41	+ + +	15/354 = 4.23	<0.001
	+ − +	5/151 = 3.31	+ + +	4/105 = 3.91	0.80
	+ + −	14/199 = 7.03	+ + +	8/146 = 5.48	0.55
	− − −	33/246 = 13.41*	+ + +	27/605 = 4.46*	<0.001

* Total of either − − − or + + + treatments.

perature exposure, hatchability fell. There was no significant difference shown between − − + (15.09) and + − − (15.82). Cold treatment given during irradiation only, + − +, increased hatchability slightly over + + + in one experiment (10.34 over 9.67), lowered it slightly in the other (3.31 and 3.91), neither difference being significant. In summary, the higher hatchability of − − − than of + + + results from protection by low temperature before and after irradiation while low temperature during exposure (1½–2 minutes) has no significant effect.

DISCUSSION

Many tests have been made on the relation of temperature differences to incidence of X-ray-induced changes in the cell, often with apparent inconsistencies in results. Those for cytogenetical effects reported before 1949 are summarized by BAKER (1949) and before 1954 by MULLER (1954). BAKER noted that, in all the experiments reporting a positive effect of temperature during irradiation, the low temperature used varied from 0° to 4°C and the high from 20° to 32°C; in experiments indicating no effect, the two temperature ranges used were 5° to 10°C and 34° to 37°C. When attention was concentrated on the relation of oxygen to X-ray-induced injury (THODAY and READ 1947), it became clear that some effects which seem to be owing to temperature differences could be explained by oxygen changes in the cell.

That the oxygen effect is not wholly responsible for temperature-radiation experimental results has been clearly demonstrated (GILES, BEATTY and RILEY 1951). Inflorescences of Tradescantia were irradiated using (1) a constant percentage of oxygen at different temperatures, (2) a constant temperature with different percentages of oxygen, and (3) various temperatures in the absence of oxygen. In (1) damage was greater at low temperatures, in (2) greater at high oxygen concentration, and in (3) less at low temperatures. Result (3) demonstrates a temperature effect *sensu stricto*, it being the reverse of (1) when oxygen was present.

Therefore, temperature and oxygen independently can influence X-ray-induced chromosome change. When. during exposure to low temperature, oxygen is available, the low temperature effect may be counteracted by that of oxygen. X-ray-induced injury thereby would be increased. In animals, the effect of extended periods of temperature change would depend upon the availability of oxygen to the cells as well as temperature conditions. An insect exposed to very low temperature for a relatively long period might have little oxygen in the cell environment if spiracles are closed and tracheal action is inhibited. X-ray-induced injury thus would be reduced. This oxygen-temperature relation seems to explain the results obtained in experiments presented in this paper.

Metaphase I tetrads at the time of exposure to X-rays have almost completely terminalized and appear stretched between centromeres and the terminal chiasmata. They break in the "stretched" dyad regions with lateral fusion of broken ends of chromatids and this fusion prevents restitution of breaks. Later meiotic

stages furnish evidence of this behavior by the presence of double fragments in division I and bridges in division II. Tension may be responsible for high frequency of breaks, lateral fusion for prevention of recovery.

METZ and BOZEMAN (1940), in discussing the high sensitivity of anaphase I chromosomes of Sciara oöcytes to X-rays, suggested that cells in which chromosomes are more actively moving or changing in form are more sensitive to X-rays than those in which chromosomes are relatively quiet. Tension was suggested (WHITING 1945) as a possible factor in sensitivity between metaphase I and prophase I (LD 50 about 400 and 12,000r respectively) in Habrobracon.

SAX (1943) found that centrifugation applied simultaneously with X-irradiation causes the broken ends of Tradescantia chromosomes to separate, thereby increasing injury. SPARROW, MOSES and DUBOW (1952) wrote, "Sensitivity appears to be related to the degree of chromosomal contraction." WOLFF and VON BORSTEL (1954) observed increased aberration frequency after postirradiation centrifugation in Tradescantia microspores and in Vicia seed. OSTER (1956) reviewed the evidence for greater sensitivity of spermatids than of mature spermatozoa in Drosophila. It has been suggested that the elongation of the spherical spermatid and chromosome movement in spermiogenesis might be the cause of the greater sensitivity. OSTER believes that this explanation is disproved by an experiment in which he irradiated males with either a rod X chromosome or a ring-shaped X. He got no difference in incidence of sex-linked lethals although in meiosis after irradiation ring chromatids should be less sensitive to stress than rods.

Does oxygen increase chromosome movement and stress? Does low temperature reduce chromosome movement and stress?

One result obtained in the present study, difficult to reconcile with the theory of tension and the facts of terminal deletion and lateral fusion to explain the extreme sensitivity of metaphase I chromosomes, is the significant protection afforded by low temperature *after* irradiation. Breakage probably has occurred before exposure; thus, if restitution has taken place, low temperature must have prevented the lateral fusion of sister chromatids. WOLFF and LUIPPOLD (1958) found that removal of oxygen or inhibition of respiration subsequent to irradiation inhibits rejoining. Either treatment allows breaks to remain open and capable of forming exchanges with breaks induced later. Does low temperature through inhibition of respiration prevent lateral fusion of broken ends of dyad chromatids and thereby permit restitution upon return to higher temperature? This seems plausible since a relative decrease in lateral fusion is a required factor for recovery or reduction of injury.

Facts which must be reconciled with the theory of stress, if it is to be maintained, are the direct relations between incidence of visible and of recessive lethal mutations with incidence of dominant lethals. After 1100r, recessive lethal rate for metaphase I is 15.2 percent, visible rate 4.35 percent and dominant lethal rate about 75 percent. On the other hand, for late prophase I, after 1100r, visible mutation rate is 0.995 percent and lethal rates are so low as to be difficult of

significant detection. If visible and some recessive lethal mutations are "point mutations," their rates should not be affected by movement or chromosome tension. However, the consistently greater sensitivity of stages in which chromosomes are under stress challenges the student of radiation effects to continue the search for a theory consistent with all facts.

SUMMARY

1. Unmated females of Habrobracon were X-irradiated with 1263 and 1444r at high and low temperature before, during, and/or after irradiation.

2. Observed was a significant lowering of lethal changes induced in oöcytes X-irradiated in first meiotic metaphase (LD 50 about 400r) at low temperatures before and after exposure as measured by hatchability of unfertilized eggs.

3. Low temperature during irradiation (1½–2 minutes) did not significantly change survival rate although there is a suggestion of a tendency toward increased lethality.

4. Theories are discussed as to the cause or causes of differential sensitivity of cells and the relation of this sensitivity to oxygen and temperature.

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ENUMERATION OF QUAD TYPES IN DIPLOIDS AND TETRAPLOIDS

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THE enumeration of different types of quad in diploids is a matter of some complexity when approached empirically (WHITEHOUSE 1942). In tetraploids complexity becomes intolerable unless more powerful techniques are used.

The first application of group theory to the enumeration of diploid and tetraploid quads was made by FISHER (1947). In the present paper FISHER's method is described, is simplified by quoting characters in terms of classes of the symmetric group, and is extended by a formula for the characters.

In describing the approach a systematic method of finding the minimal number and position of crossovers that will account for a given quad is presented.

Mathematical theorems are stated and proved in the last section.

Types of quad and crossing over: In a diploid cell undergoing meiosis each of the four strands of a tetrad usually go to a separate cell. The four resulting haploid cells form a quad.

Exceptions occur in *Neurospora tetrasperma* and in *Podospora anserina* (RIZET and ENGELMANN 1949). In both of these the tetrad strands divide with overlapping spindles to produce binucleate quads. Sister strands from the third division find themselves in different cells. In *N. tetrasperma* the spindles of the second meiotic division overlap and the strands in a cell of the quad are second cousins in the sense that their nearest common ancestor nucleus is the zygote. In *P. anserina* the second meiotic spindles do not overlap, and strands in the same quad cell are first cousins. These and other exceptions can be considered derived types.

If the four strands of a tetrad or cells of a quad are numbered one through four there are $4! = 24$ different strand orders. Where only one locus is followed these 24 strand orders cannot all be distinguished from one another because the alleles on strands one and two are identical, being derived from the same gamete, and similarly with the alleles on strands three and four. For one locus and two alleles there are in fact $4!/2!2! = 6$ distinguishable orders. These six orders can each be changed by permutations, thus order *AAaa* is transformed by (2,3) to *AaAa*. Again, $Aa\,Aa \xrightarrow{(2,3,4)} AaaA$; the permutation (2,3,4) signifying that the letter "*a*" in second position goes to position three, the letter "*A*" in third position goes to position four and the last letter "*a*" to position two, while the first letter "*A*" stays put. There are in all a group of $4! = 24$ such permutations. They include a cycle of length one, which leaves the order unchanged, six cycles of length two, three double cycles of length two, eight cycles of length three, and six cycles of length four; these are listed in column 3 of Table 1 and appear in Table 4. Each

TABLE 1

Group table

Stability subgroup and five cosets	Stability subgroup	Permutations of the group	Minimum crossovers	Gene orders
( )	( )	( )		*A*
( )	(1,2)	(1,2)		*A*
( )	(3,4)	(3,4)		*a*
( )	(1,2) (3,4)	(1,2) (3,4)		*a*
(2,3)	( )	(2,3) =	(2,3)	*A*
(2,3)	(1,2) =	(1,3,2) =	(1,3) (2,3)	*a*
(2,3)	(3,4) =	(2,3,4) =	(2,4) (2,3)	*A*
(2,3)	(1,2) (3,4) =	(1,3,4,2) =	(1,3) (2,4) (2,3)	*a*
(1,4)	( )	(1,4) =	(1,4)	*a*
(1,4)	(1,2) =	(1,2,4) =	(2,4) (1,4)	*A*
(1,4)	(3,4) =	(1,4,3) =	(1,3) (1,4)	*a*
(1,4)	(1,2) (3,4) =	(1,2,3,4) =	(2,4) (1,3) (1,4)	*A*
(2,4)	( )	(2,4) =	(2,4)	*A*
(2,4)	(1,2) =	(1,4,2) =	(1,4) (2,4)	*a*
(2,4)	(3,4) =	(2,3,4) =	(2,3) (2,4)	*a*
(2,4)	(1,2) (3,4) =	(1,4,3,2) =	(2,3) (1,4) (2,4)	*A*
(1,3)	( )	(1,3) =	(1,3)	*a*
(1,3)	(1,2) =	(1,2,3) =	(2,3) (1,3)	*A*
(1,3)	(3,4) =	(1,3,4) =	(1,4) (1,3)	*A*
(1,3)	(1,2) (3,4) =	(1,2,3,4) =	(2,3) (1,4) (1,3)	*a*
(1,3) (2,4)	( )	(1,3) (2,4) =	(1,3) (2,4)	*a*
(1,3) (2,4)	(1,2) (3,4) =	(2,3) (1,4) =	(2,3) (1,4)	*a*
(1,3) (2,4)	(1,2) =	(1,4,2,3) =	(2,3) (2,4) (1,4)	*A*
(1,3) (2,4)	(3,4) =	(1,3,2,4) =	(1,3) (1,4) (2,4)	*A*

permutation produces a different strand order, and if the original order is taken as 1,2,3,4, then the 24 permutations produce the 24 strand orders listed in column 1 of Table 2. Each row of Table 1 corresponds to a row of Table 2, but the strand orders in Table 2 are produced by the inverse of the permutation in the corresponding row of Table 1. Writing the orders produced by the inverse of the permutation has been necessary in order to simplify the use of Table 2.

Crossovers and quad type: Although only six different orders of quad are distinguishable for a single gene, when several loci are followed all 24 strand orders must be considered. These 24 strand orders are listed in the 24 rows of Table 2. The relations of crossovers to the group of permutations in Table 1 and the use of Table 2 will be explained below.

For a locus with no crossovers between it and the centromere which therefore has the gene order *AAaa*, sister strand crossovers, i.e. (1,2), (3,4) and (1,2) (3,4), leave the quad type unaltered. Together with no crossover ( ), they constitute the stability subgroup at *AAaa*. Each of the five other gene orders besides *AAaa*

TABLE 2

Table of transformations of orders

Strand order	Code nos. for strand order	Type of crossover (2,3)	(1,4)	(2,4)	(1,3)	(1,4)(2,3)	(1,3)(2,4)	Gene orders
1234	1.1	2.1	3.1	4.1	5.1	6.1	6.2	*A*
2134	1.2	5.2	4.2	3.2	2.2	6.4	6.3	*A*
1243	1.3	4.3	5.3	2.3	3.3	6.3	6.4	*a*
2143	1.4	3.4	2.4	5.4	4.4	6.2	6.1	*a*
1324	2.1	1.1	6.1	4.3	5.2	3.1	3.4	*A*
2314	2.2	5.1	4.4	6.3	1.2	3.3	3.2	*a*
1423	2.3	4.1	5.4	1.3	6.4	3.2	3.3	*A*
2413	2.4	6.2	1.4	5.3	4.2	3.4	3.1	*a*
4231	3.1	6.1	1.1	4.2	5.3	2.1	2.4	*a*
4132	3.2	5.4	4.1	1.2	6.3	2.3	2.2	*A*
3241	3.3	4.4	5.1	6.4	1.3	2.2	2.3	*a*
3142	3.4	1.4	6.2	5.2	4.3	2.4	2.1	*A*
1432	4.1	2.3	3.2	1.1	6.2	5.4	5.1	*A*
2431	4.2	6.4	1.2	3.1	2.4	5.2	5.3	*a*
1342	4.3	1.3	6.3	2.1	3.4	5.3	5.2	*a*
2341	4.4	3.3	2.2	6.1	1.4	5.1	5.4	*A*
3214	5.1	2.2	3.3	6.2	1.1	4.4	4.1	*a*
3124	5.2	1.2	6.4	3.4	2.1	4.2	4.3	*A*
4213	5.3	6.3	1.3	2.4	3.1	4.3	4.2	*A*
4123	5.4	3.2	2.3	1.4	6.1	4.1	4.4	*a*
4321	6.1	3.1	2.1	4.4	5.4	1.1	1.4	*a*
3412	6.2	2.4	3.4	5.1	4.1	1.4	1.1	*a*
4312	6.3	5.3	4.3	2.2	3.2	1.3	1.2	*A*
3421	6.4	4.2	5.2	3.3	2.3	1.2	1.3	*A*

can be produced by four permutations which constitute a coset of the stability subgroup. The stability subgroup and its five cosets are given in column 3 of Table 1. In genetic terms a reciprocal crossover is represented by a permutation of length two, and the six basic crossover types, corresponding to six distinguishable gene orders, are listed in column 1 of Table 1. They can each occur alone or following any of the three sister strand crossovers which are listed in column 2 of Table 1. In the fourth column of Table 1 each permutation has been written as a minimum sequence of crossovers, sister strand crossovers being forbidden.

For example, rows 5, 6, 7 and 8 of Table 1 represent permutations giving the gene order *AaAa*. Row six in particular consists of the crossover (2,3) applied after a sister strand crossover (1,2). These give the permutation $(2,3)(1,2)=(1,3,2)$ which is equivalent to $(1,3)(2,3)$ or a crossover (2,3) followed by a crossover (1,3). It can be seen that any permutation can be produced by no more than three crossovers. Table 2, in mathematical terms, is that portion of the multiplication table of the group of permutations which includes as multipliers only the six permutations representing the six basic crossover patterns.

Table 2 has been made more readable by giving the 24 strand orders code numbers consisting of one through six before a decimal point and one through four after the decimal. The six numbers before the decimal correspond to the six gene orders and are the same as have been used previously (PAPAZIAN and LINDEGREN 1960), the four numbers after the decimal specify one of the four strand orders giving that particular gene order.

The way in which Table 2 can be used to find the minimum number of crossovers which would account for a given quad will be illustrated by an example.

If we represent the alleles from one parent as "*A*"s and from the other parent "*a*"s then the fourth quad from the bottom of Table 1 of LINDEGREN (1942) can be represented by the matrix:

$$\begin{matrix} a & A & (A) & A & A \\ a & a & (A) & a & a \\ A & A & (a) & A & a \\ A & a & (a) & a & A \end{matrix}$$

where successive columns are successive loci and the column with brackets is the centromere locus. If code numbers are used for the gene order of each column and (1) for the centromere this quad is 62(1)24.

Let us arbitrarily call the strand order at the first locus 6.1. Then we have to get from 6.1 to a strand order representing gene order 2. From Table 2 row 21 it is found that the only 2. entry is 2.1 in the column headed (1,4) thus a crossover between strands one and four is required in the first interval. From 2.1 to get to a 1., we need (2,3) giving 1.1 and when the process is completed we have $6.1 \xrightarrow{(1,4)} 2.1 \xrightarrow{(2,3)} (1.1) \xrightarrow{(2,3)} 2.1 \xrightarrow{(2,4)} 4.3$. The relation between successive crossovers is therefore 4 strand:2 strand:3 strand and not 4:2:4 as misprinted in LINDEGREN (1942).

Multiplying out the successive crossover permutations (1,4)(2,3)(2,3)(2,4)= (142) and $6.1 \xrightarrow{(1,4,2)} 4.3$.

We could have started with strand orders 6.2, 6.3 or 6.4 in which case the crossovers and orders would have been:

$$6.2 \xrightarrow{(2,3)} 2.4 \xrightarrow{(1,4)} (1.4) \xrightarrow{(1,4)} 2.4 \xrightarrow{(1,3)} 4.2$$

$$6.3 \xrightarrow{(2,4)} 2.2 \xrightarrow{(1,3)} (1.2) \xrightarrow{(1,3)} 2.2 \xrightarrow{(1,4)} 4.4$$

$$6.4 \xrightarrow{(1,3)} 2.3 \xrightarrow{(2,4)} (1.3) \xrightarrow{(2,4)} 2.3 \xrightarrow{(2,3)} 4.1$$

All four patterns of crossing over are equivalent and retain the same relations between successive crossovers. When double crossovers are involved in an interval, two alternative minimum crossover patterns are always possible.

Some chromosomal entanglements can be unravelled by the above considerations. In the chromosomal heterozygotes of SCHWARTZ (1953) the effect of crossing over between a ring and a rod chromosome were described. In such a situation the result of a three-strand double crossover, say (2,3) (2,4), can be found as follows.

Let the original order of strands in the tetrad be 1234. This order will be converted by (2,3) to 1324 and by (2,4) to 1342; the total change will be from 1234 to 1342, but if strands 3 and 4 constitute a ring the ends must be joined; this can be written as shown in Diagram 1 where the first meiotic division I, represented by the central solid line, cuts across a dotted line which will cause a bridge at this division. Similarly at the second meiotic division II a solid line is cut on one side but not on the other, and there will be one bridge in one of the second meiotic divisions.

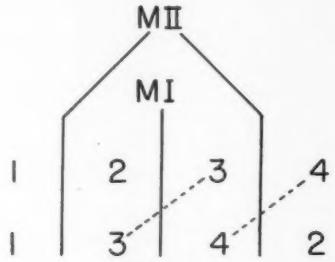

DIAGRAM 1.

Enumeration of quad types: Since the number of equivalent quads is greatest when they are unordered these will be considered first. Formulae for ordered quads immediately follow.

Unordered quads: Two unordered quads may be considered *equivalent* if there is a permutation of the group which changes one into the other. In this sense all unordered quads of one locus are equivalent. If, however, we now consider two loci, the $6^2 = 36$ orders are not all equivalent.

In fact there are three nonequivalent types, the parental and nonparental ditypes and a tetratype. These three types cannot be changed one into another by any permutation of the group.

When three or more loci are considered, certain quad types such as $\begin{matrix} AAa \\ AaA \\ aAA \\ aaa \end{matrix}$ and $\begin{matrix} AAA \\ Aaa \\ aAa \\ aaA \end{matrix}$ can be changed one into the other by an exchange of allele symbols (BENNETT 1956; PAPAZIAN 1957) represented by (Aa). If quads that can be changed into one another by an exchange of allele symbols are considered equivalent, the number of different types of quad becomes what FISHER has termed the "modes of formation."

The calculation of equivalent permutations and hence of quad types for any l loci is summarized in Table 3.

Explanation of the calculation of Table 3: FISHER (1950) has given a formula for the number of inequivalent types (see also SPEISER (1927) theorem 102) *viz.*

$$\frac{1}{g} \sum_{\gamma}^{g} x_{\gamma} \qquad (5)$$

TABLE 3

Characters for diploid quads and enumeration formulae

Class c	d	Order (group for ordered quads) g_c	$k_{c,d}$	Character $\chi_{c\times d}=6k_{c,d}/g_c$	Order (subgroup for ordered quads)
(1^4)	(1^2)	1	1	6	1
(1^22)	(1^2)	6	2	2	2
(2^2)	(1^2)	3	1	2	3
(1 3)	(1^2)	8	0	0	0
(4)	(1^2)	6	0	0	2
Total of first five		24			8
(1^4)	(2)	1	0	0	1
(1^22)	(2)	6	0	0	2
(2^2)	(2)	3	2	4	3
(1 3)	(2)	8	0	0	0
(4)	(2)	6	2	2	2
Total		48			16

	Unordered quads		Ordered quads	
		(3)		(4)
Without permutation of allele symbols (using only first five rows)	$\frac{1}{24}(6^l+9.2^l)$		$\frac{1}{8}(6^l+5.2^l)$	
		(1)		(2)
With permutation of allele symbols	$\frac{1}{48}(6^l+3.4^l+15.2^l)$		$\frac{1}{16}(6^l+3.4^l+7.2^l)$	

(1) and (2) were published by FISHER (1950). (1), (3) and (4) were derived by another method in PAPAZIAN 1951. The division of the fraction before each formula is the appropriate total in columns 2 or 5. The terms within brackets are the lth powers of the characters in column 4 each multiplied by the total number of permutations, obtained from columns 2 or 5, having the particular character.

where g is the *order* of the permutation group, i.e. the number of permutations in it including the identity; and χ_γ is the *character* of the group element γ which is defined as the number of quads which are unaltered by the permutation.

All permutations of a certain *class* have the same character. For the symmetric group of all permutations of the positions, it can be shown that the class to which a permutation belongs is determined by the lengths of the cycles into which it decomposes, e.g. thus (2,3,4), a cycle of length 3, equals (1)(2,3,4). The symbol (1 3) denotes its class. Likewise (2^2) is the class of permutations with two cycles of length 2. The permutations of each class are listed in Table 4.

As the character χ_γ is the same for all the g_c permutations γ in a class c, the formula (5) for the number of types can be rewritten

$$\frac{1}{g}\sum_c g_c \quad \chi_c \tag{6}$$

If χ_c is the character for permutations of quads for one locus, the character for l loci is clearly $\chi^l{}_c$. Hence the number of types for l loci is

$$\frac{1}{g}\sum_c g_c \quad \chi^l{}_c \tag{7}$$

TABLE 4

Classes of the symmetric group on 4 symbols

Class, c	Permutations as in column 3, Table 1	Order, g_c
(1^4)	$(1)(2)(3)(4) = (\ ) =$ identity	1
$(1^2 2)$	$(1,2)(3)(4) = (1,2), (1,3), (1,4), (2,3), (2,4), (3,4)$	6
(2^2)	$(1,2)(3,4), (1,3)(2,4), (1,4)(2,3)$	3
$(1\ 3)$	$(1,2,3), (1,2,4), (1,3,2), (1,3,4), (1,4,2), (1,4,3), (2,3,4), (2,4,3)$	8
(4)	$(1,2,3,4), (1,2,4,3), (1,3,4,2), (1,3,2,4), (1,4,2,3), (1,4,3,2)$	6
		24

For each class c, χ_c is readily found by taking any fixed permutation γ in c and counting how many of the six gene orders are left fixed by it. The values are given in the first five rows of column 4 of Table 3 and equation (7) yields the formula (3) for the number of unordered tetrads without permutation of the gene symbols.

If permutation of allele symbols is allowed, and d is a class of this group, then a class $c \times d$ of the combined group consists of all pairs of a permutation of c combined with a permutation of d.

With two allele symbols, there are two classes, each consisting of a single permutation, *viz.*

Class, d	Permutations	
(1^2)	$(A)(a)=(\ )$	(first five rows of Table 3)
(2)	(Aa)	(last five rows of Table 3)

Replacing c by $c \times d$ in (7) and taking g=48, we have (1), all ten rows of Table 3 are relevant here.

For ordered quads, the group of all permutations of positions is replaced by a subgroup of order 2^3 of permutations within and between the first and last pairs of positions 12,34; these are permutations corresponding to the orientation of the first and second meiotic division spindles respectively and are not significant with regard to crossing over. The last column of Table 3 gives the numbers of elements of the subgroup in the various classes of the group. By using them in place of g_c in (7), we obtain the formulae (2) and (4) for the ordered quads.

In more complex cases, when the evaluation of the character by counting the invariants becomes very tedious and confusing, the formula

$$\chi_c \times d = \frac{t\, k_{c,d}}{g_c} \tag{8}$$

proved in the last section, is useful; t is the number of tetrads; $k_{c,d}$ is the number of position permutations of class c which, for any fixed quad and any fixed permutation of allele symbols of class d, will achieve the same transformation of this quad. For example, to find $k_{(4),(2)}$ given in the last line of Table 3, consider the effect of (Aa), which is of class d=(2), on the quad *AAaa*,

$$AAaa \xrightarrow{(Aa)} aaAA$$

This can be achieved by position permutations (1324) or (1423) of class (4) but by no others of this class. Hence $k_{(4),(2)} = 2$.

Tetraploid quads

For one locus with four allele symbols A,B,C,D, there are $t=8!\ (2!)^4 = 2520$ quads comprised of the symbols $AABBCCDD$ written in all orders. The unordered quads have a group of $4!2^4$ permutations within and between the pairs of positions 12,34,56,78. The *ordered* quads admit a group of 2^7 permutations indicated by Diagram 2.

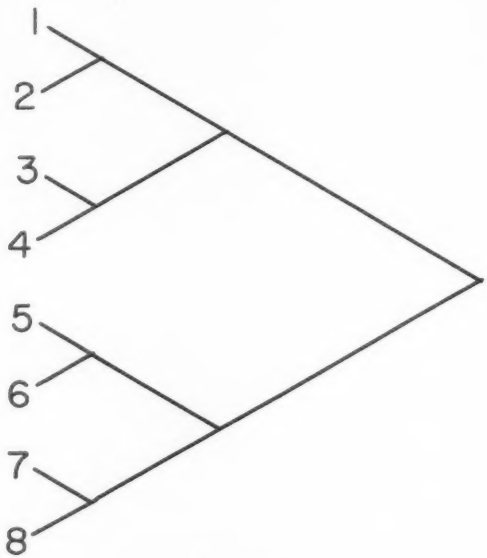

DIAGRAM 2.

These correspond, from left to right, to the order of two alleles within a quad cell, the orientation of the second meiotic spindles and the orientation of the first meiotic spindle.

It is convenient first to calculate the character for the group of all 8! permutations of positions. There is a group of 4! permutations of the allele symbols, if such are allowed, with classes d as given in Table 3. The calculations are set out in Table 5. The number $k_{c,d}$, e.g. for $c = (4^2)$ and $d = (4)$, is found as the number of permutations of positions of class (4^2) which will produce the same effect upon any one fixed tetrad, e.g. $AABBCCDD$, as the permutation $(ABCD)$ of class (4) of allele symbols:

$$AABBCCDD \xrightarrow{(ABCD)} BBCCDDAA$$

The only position permutations of class (4^2) which will achieve this are clearly

(1753)(2864)	(1754)(2863)
(1853)(2764)	(1854)(2763)
(1763)(2854)	(1764)(2853)
(1863)(2754)	(1864)(2753)

Hence $k_{(4^2),(4)} = 8$.

TABLE 5

Character for tetraploid quads with four allele symbols

Class (gene symbols) c	d	Order of c g_c	$k_{c,d}$	Character $\chi_{c\times d} = \frac{2520k_{c,d}}{g_c}$	Subgroups for Unordered	Subgroups for Ordered
(1^8)	(1^4)	1	1	2520	1	1
(1^62)		28	4	360	4	4
(1^42^2)		210	6	72	18 × 1	10 × 1
(1^22^3)		420	4	24	28	12
(2^4)		105	1	24	25	17
(1^42^2)	(1^22)	210	2	24	18	10
(1^22^3)		420	4	24	28	12
(2^4)		105	2	48	25 × 6	17 × 6
(1^44)		420	2	12	12	4
$(1^22\ 4)$		2520	4	4	24	8
(2^24)		1260	2	4	36	28
(1^23^2)	$(1\ 3)$	1120	4	9	32	0
$(2\ 3^2)$		1120	4	9	32 × 8	0 × 8
(1^26)		3360	4	3	32	0
$(2\ 6)$		3360	4	3	32	0
(4^2)	(4)	1260	8	16	60 × 6	28 × 6
(8)		5040	8	4	48	16
(2^4)	(2^2)	105	4	96	25	17
(2^24)		1260	8	16	36 × 3	28 × 3
(4^2)		1260	4	8	60	28

	Unordered	Ordered
Without permutation of allele symbols (using only the first five rows)	$\frac{1}{2^44!}(2520^l + 4.360^l + 18.72^l + 53.24^l)$	$\frac{1}{2^7}(2520^l + 4.360^l + 10.72^l + 29.24^l)$
With permutation of allele symbols	$\frac{1}{2^4(4!)^2}(2520^l + 4.360^l + 75.96^l + 18.72^l + 150.48^l + 329.24^l + 468.16^l + 72.12^l + 512.9^l + 180.8^l + 648.4^l + 512.3^l)$	$\frac{1}{2^74!}(2520^l + 4.360^l + 51.96^l + 10.72^l + 102.48^l + 161.24^l + 252.16^l + 24.12^l + 84.8^l + 352.4^l)$ (as given by FISHER (1950))

The calculations are similar to those for the diploid in Table 3, except that, for simplicity, classes with zero character are omitted and hence the totals of the fifth and sixth columns are not the orders of the respective groups. Columns 5 and 6 give the orders of the classes, $c\times d$, as the number of position permutations (for unordered or ordered) belonging to class c times the order of class d.

TABLE 6

Character for tetraploid quads with two allele symbols

Class c	Class d	Order of c g_c	$k_{c,d}$	Character $\chi_{c\times d}=\frac{70k_{c,d}}{g_c}$	Subgroups for Unordered	Subgroups for Ordered
(1^81)	(1^2)	1	1	70	1	1
(1^62)	(1^2)	28	12	30	4	4
(1^44)	(1^2)	420	12	2	12	4
(1^42^2)	(1^2)	210	42	14	18	10
$(1^22\ 4)$	(1^2)	2520	72	2	24	8
(1^22^3)	(1^2)	420	36	6	28	12
(1^23^2)	(1^2)	1120	64	4	32	0
(4^2)	(1^2)	1260	36	2	60	28
(2^24)	(1^2)	1260	36	2	36	28
(2^4)	(1^2)	105	9	6	25	17
(8)	(2)	5040	144	2	48	16
(4^2)	(2)	1260	72	4	60	28
(2^24)	(2)	1260	144	8	36	28
(2^4)	(2)	105	24	16	25	17
(2 6)	(2)	3360	192	4	32	0

	Unordered	Ordered
Without permutation of allele symbols (using only first ten rows)	$\frac{1}{2^44!}(70^l+4.30^l+18.14^l+53.6^l+32.4^l+132.2^l)$	$\frac{1}{2^7}(70^l+4.30^l+10.14^l+29.6^l+68.2^l)$
With permutation of allele symbols	$\frac{1}{2^54!}(70^l+4.30^l+25.16^l+18.14^l+36.8^l+53.6^l+124.4^l+180.2^l)$	$\frac{1}{2^8}(70^l+4.30^l+17.16^l+10.14^l+28.8^l+29.6^l+28.4^l+84.2^l)$

The calculations are analogous to those in Tables 3 and 5.

In Table 6 the calculations are given for another example, that of the duplex tetraploid having only two allele symbols *AAAAaaaa*. This would be typical for autotetraploids.

In organisms like *P. anserina* and *N. tetrasperma* there is nothing corresponding to an unordered quad. The enumeration of quad types in these organisms is the same as that for ordered quads in standard diploids if a distinction is made between alleles in coupling and in repulsion phase. If such a distinction is not made the number of quad types is given by $(3^l+1)/2$. A corresponding enumeration for tetraploids appears complex and will not be made here.

The relation of crossovers to quad types in tetraploids has been analyzed for one locus by LEUPOLD (1956) using different algebraic methods.

With independent assortment of all genes the relative frequencies of different quad types and the probability of getting identical quads has been calculated for the diploid by PAPAZIAN (1952).

Numerical values of the various enumerations for $l = 1, 2, 3, 4, 5$ and 6 loci are given in Table 7.

MATHEMATICAL THEORY

Characters of permutation groups

Theorem I: If G is a transitive group of permutations of a set T with *stability* or *isotropy* group H at $t_0 \in T$, then the character of G is

$$\chi_c = \frac{g\, h_c}{h\, g_c}$$

where χ_c is the value of the character for the g_c elements of the conjugation class c of G, g and h are the orders of G and H, and h_c is the number of elements common to c and H.

Proof: T is isomorphic to the left coset space G/H under the correspondence

$$t \longleftrightarrow \tau H$$

where τ is any element of G such that $\tau t_0 = t$. Hence

$$\gamma\, t = t \qquad \gamma \epsilon c \subset G$$

if and only if

$$\gamma\, \tau H \subset \tau H$$

if and only if

$$\gamma\, \tau \epsilon\, \tau H$$

if and only if

$$\tau^{-1} \gamma\, \tau \epsilon\, H$$

TABLE 7

Numerical values for various enumerations of quad types

		Number of loci					
		1	2	3	4	5	6
Diploid							
(2N)	U P	1	3	11	48	236	1,248
	U	1	3	12	60	336	1,968
	O P	2	7	29	136	692	3,712
	O	2	7	32	172	992	5,872
Tetraploid							
(4N)	U P	5	910	1,767,188	4,383,958,398	...	....
	O P	8	2,538	5,290,732	13,151,034,348	...	....
	U	17	18,210	42,181,392	....	...	....
	O	42	54,072	125,420,292	....	...	....
Duplex							
(2N+2N)	U P	3	33	836	38,842	2,363,958	157,724,468
	U	3	39	1,341	72,970	4,656,328	314,331,104
	O P	5	72	2,231	112,926	7,040,150	472,399,692
	O	5	92	3,791	216,194	13,933,640	942,512,912
N. tetrasperma type							
(2N)		2	5	14	41	122	365

O = ordered.
U = unordered.
P = allowing permutation of allele symbols.

Since there are clearly g/g_c elements $\tau \epsilon G$ which conjugate γ to a fixed element of its class c, there are gh_c/g_c elements in G which conjugate γ into H. These form

$$x_c = \frac{g\, h_c}{h\, g_c}$$

cosets τH corresponding to elements $t \epsilon T$ which are left fixed by γ.

Theorem II: If H is a normal subgroup of N and N is a subgroup of G

$$H \underset{\text{normal}}{\subset} N \subset G$$

then the character $\chi_{c,d}$ of the transformation group $G \times N/H$ of the left coset space G/H,

$$\tau H \longrightarrow \gamma \tau H \nu \qquad \tau H \epsilon \quad G/H, \qquad \gamma \epsilon G, \qquad \nu H = H \nu \epsilon N/H,$$

is

$$\chi_{c,d} = \frac{g\, f_{c,d^{-1}}}{h\, g_c\, n_{d^{-1}}} = \frac{t\, k_{c,d^{-1}}}{g_c} \tag{1}$$

where c is the class of G to which γ belongs and has g_c elements,

d is the class of N/H to which νH belongs,

$\chi_{c,d}$ is the value of the character for the class $c \times d$ of $G \times N/H$,

g and h are the orders of G and H,

$f_{c,d^{-1}}$ is the number of elements of $c \cap Hd^{-1}$,

d^{-1} is the class of N/H comprised of the inverses of the elements of the class d,

$Hd^{-1} \subset N$ is the union of the cosets in d^{-1},

$n_{d^{-1}}$ is the order of the class d^{-1}.

$t = g/h =$ order of G/H

$k_{c,d^{-1}} = f_{c,d^{-1}}/n_{d^{-1}}$

Proof: The coset τH is mapped into itself by $(\gamma \times \nu H) \epsilon (G \times N/H)$ if and only if

$$\gamma(\tau H)\nu \epsilon \tau H \qquad \gamma \epsilon G, \quad \tau H \epsilon GH/ \qquad \nu H \epsilon N/H$$

if and only if

$$\gamma \tau \nu \epsilon \tau H$$

if and only if

$$\tau^{-1} \gamma \tau \epsilon H \nu^{-1} \tag{2}$$

Since $\tau^{-1}\gamma\tau \epsilon c$, the number of $\tau \epsilon G$ which conjugate γ into $H\nu^{-1}$ is gk/g_c, where $k = k_{c,d^{-1}}$ is the number of elements in $c \cap H\nu^{-1}$. Remembering that there are h such τ in each coset τH, we have

$$\chi_{c,d} = \frac{g\, k}{h\, g_c}$$

But since $c \cap Hd^{-1}$ clearly has $n_{d^{-1}}\, k$ elements, $f_{c,d^{-1}} = n_{d^{-1}}\, k$ and equation (1) follows.

SUMMARY

Formulae for numbers of quad types in diploids, tetraploids with four or two allele symbols, and *N. tetrasperma* type are obtained by use of the theory of group characters. Both ordered and unordered types with or without interchange of gene symbols are enumerated.

A multiplication table of crossover permutations is given.

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THE CYTOGENETICS OF "FLAKED," A VARIEGATION IN TOMATO AFFECTING TWO CELL LAYERS[1]

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THERE are at least six current explanations of variegation: plastid differences; position effect (LEWIS 1950) in some manner related to heterochromatin; controlling units including *Ac-Ds* (MCCLINTOCK 1950), *Mp* (BRINK and NILAN 1952), and *En* (PETERSON 1960); somatic crossing over (STERN 1936; JONES 1937) revealing hidden heterozygosity; a gene for stickiness of the chromosomes (BEADLE 1932); and irregular distribution in the soma of a ring or rod chromosome. The flaked variegation appears to be due to irregular distribution of a small chromosome fragment.

Somatic lagging and loss occur when a ring or rod becomes dicentric; this may also result when a fragment is telocentric. Like ring chromosomes, telocentrics may break or double their size. The functioning of a centromere may be either weak or strong. In general, a chromosome with a very short arm has a weakly functioning centromere; therefore bridge loss tends to occur (PTASHNE 1960). An extra telocentric fragment has been described as inducing variegation in maize (RHOADES 1940) and in wheat (SEARS 1952b).

A ring chromosome causing variegation may be part of a normal chromosome complement (MCCLINTOCK 1932, 1938) or it may be present in addition to a normal complement (FROST, LESLEY and LOCKE 1959).

A chromosome fragment with a centromere at or near one end is the cause of variegation in maize, wheat, tobacco (CLAUSEN 1930) and stock. The fragment producing variegation may be either a ring or a rod and it may be as large as half a chromosome or so small as to be barely perceptible. It seems possible that weakly staining minute fragments may account for other unsolved cases of variegation.

MATERIALS AND METHODS

Seeds of the tomato variety Canary Export were soaked in a solution containing the radioactive isotope P^{32} as phosphate. The initial activity per seed was 10.9μC (LESLEY and LESLEY 1956). An R_1 plant which was slow-growing and nearly sterile but otherwise like the parent type produced 11 plants from self-pollination. Eight of these R_2 plants were moderately fruitful. The other three

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plants were less fruitful and included a new phenotype 54.27G1, which was termed "flaked."

The chromosomes were studied in aceto-orcein smears or, with the best results, in acetocarmine by the Walters method (BROWN 1949). For genetic study inbreeding for four generations and outcrossing with linkage testers were employed. The common tomato is normally self-pollinating. For better control, unless otherwise noted, all plants were hand-pollinated and, if emasculated in the field, protected with paper bags.

Description of flaked: The name flaked was suggested by the external appearance of the leaves. They are composed of small, nearly white flake-like areas surrounded by normal green tissue (Figure 1). The "white" areas usually have a green tinge due to underlying green tissue and the term "white" is only relative. White flakes are scattered irregularly and are more conspicuous on the upper surface of the lamina. The calyx may be variegated. A similar greenish white variegation occurs on the stem but consists of longitudinal stripes varying in width and length but often clearly defined. The unripe fruit has green and white sectors extending from basal to distal end, varying in width, and resembling the stem in arrangement. Ripening is abnormally slow, and as it proceeds, the white sectors, which are usually narrower than the green, fail to form lycopene in the outer cortex so that the ripe fruit is striped with red and nonred sectors. The skin color of the fruit is not affected. The cotyledons usually are slightly variegated but less so than the first leaves.

Flaked plants are smaller and have smaller leaves than normal plants of the variety in which the mutation originated. As a rule the greater part of the leaf and stem surface is normal green. The more extensive the white tissue the smaller the leaf. Leaflets which are predominately white may be reduced sometimes to mere vestiges. The lamina is sometimes distorted due to the slower growth of the white tissue. The white stem stripes are slightly sunken. The fruit is malformed if wide white sectors are present.

Variegation of another kind occurs in which the epidermal hairs are absent. Normal plants are hairy, having the trichomes and glandular hairs typical of the parent type. The epidermis of flaked is usually composed of hairy and of glabrous areas which are without trichomes and glandular hairs; the hairy areas tend to be more extensive. The pattern is similar to that of the green and white variegation. The two kinds of variegation seem to occur independently so that the four combinations, green hairy, green glabrous, white hairy, white glabrous, are found on the stems and leaves (Figure 2). Occasionally a whole shoot occurs of which the epidermis of stem, leaves, and flower is glabrous (Figure 3). The underlying tissue has the usual green and white variegation. The leaves and flowers tend to be small and the anthers are dialytic due to the absence of interlacing hairs.

The extent of white and of glabrous tissue varies in different parts of the same plant. A wholly glabrous shoot conspicuous by its glossy appearance occurs on about one fifth of the unpruned flaked plants. Some shoots appear to be wholly hairy but close examination discloses small glabrous stripes. The amount of varie-

FIGURE 1.—Leaf of flaked tomato showing green and white variegation. Lamina more distorted on right side.

gation sometimes differs considerably in sibs from selfing a variegated plant. The white areas especially may be large and the lamina distorted or small and inconspicuous. Figure 1 shows a moderately variegated leaf.

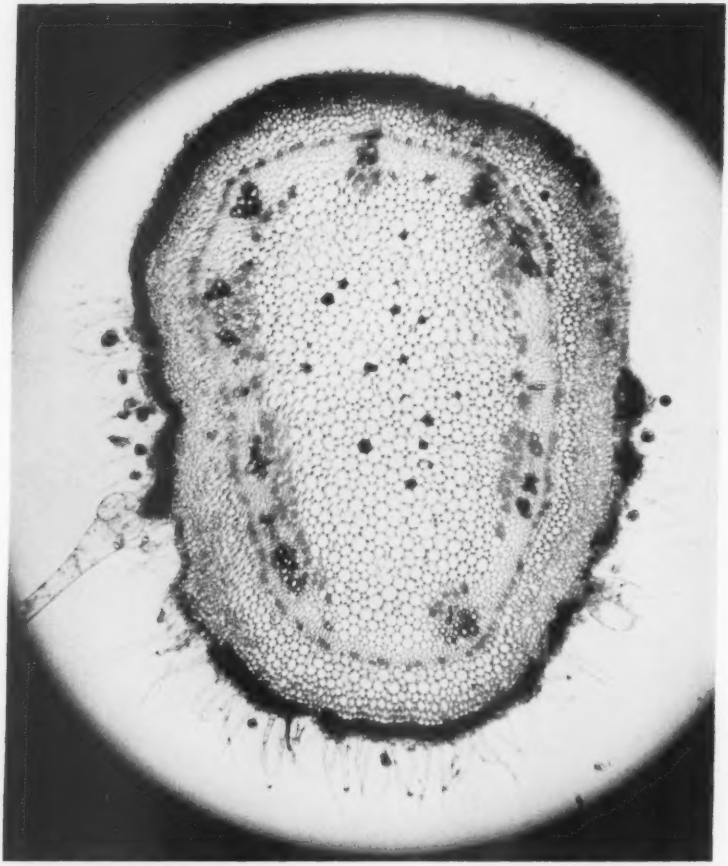

FIGURE 2.—Section of fresh stem of flaked showing variegation in chlorophyll and hairiness in outer cortex and epidermis, and the four combinations green (dark tissue) hairy, green glabrous, nongreen hairy, and nongreen glabrous.

Flaked is male fertile but subnormal in the amount of stainable pollen and is female fertile although it produces less fruit and seed from self-pollination than a normal plant. Even glabrous shoots produce some stainable pollen but no fruit even after the application of normal pollen. The dialytic staminal column partially accounts for this unfruitfulness. The dialytic sparsely hairy *dl* mutant described by RICK (1956) produced a normal crop only when artificially self-pollinated.

In sections of fresh leaves of flaked (Figure 4) the normal green palisade cells

FIGURE 3.—Shoot of flaked tomato with green and white variegation, and glabrous lateral shoot (right).

of the upper surface are absent in white areas and are more or less completely replaced by nongreen spongy tissue. The spongy tissue of the lower surface may also be deficient in chlorophyll. Under the white stem stripes the cell layers of the outer cortex contain no chlorophyll.

Flaked shoots were grafted on two normal plants and a normal shoot on flaked. The scions and stocks were unchanged, indicating no graft transmission and that flaked is not due to a virus disease.

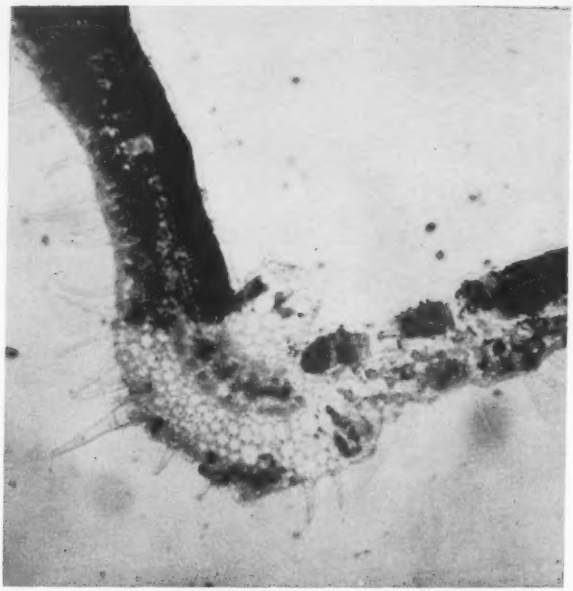

FIGURE 4.—Section of fresh leaf of flaked with palisade layer to one side of midrib interrupted by nongreen tissue.

Cytology: The original flaked R_2 plant, 54.27G1, was small and had very short internodes. Its R_2 sibs were rather pale green and of reduced fertility but were not variegated or dwarfed. That 54.27G1 was unfruitful was not surprising since it was cytologically very abnormal. The p.m.c. contained 12 pairs of chromosomes and one small fragment or nine pairs, two trivalents and one small fragment at diakinesis and M1. Rarely a ring of six was found. It is evident that the two members of one pair had undergone reciprocal translocation with two different chromosomes. Since a fragment was formed in a diploid (12 pair) R_1 plant, the chromosome of which the fragment was a part must have been deficient. The fragment was present in many p.m.c. It therefore presumably had an attachment constriction. At A1 it often lagged and was lost like other unpaired chromosomes in meiosis. A bridge was sometimes found at A1 indicating that an inversion was present as well. Since the bridge was often complex the inversion was probably in one of the chromosomes that forms a trivalent.

The variegated R_2 plant produced seeds from pollination with a normal plant. Plants from this backcross were nonvariegated. The only plant from selfing was sterile and variegated in the same manner as its parent. It was cytologically similar also, but was much more vigorous and less dwarfed than 54.27G1. It produced no progeny.

An F_2 from 54.27G1 by a normal plant of the same variety contained 14 plants of various types. One was albino, another resembled the gene mutant wiry (*w*$_s$) but none was flaked. A nonflaked F_2 plant was pollinated by 54.27G1. The resulting family 56.6 varied considerably in appearance, fruitfulness, and cytological condition. Two plants resembled the wiry mutant. It is evident from Table

TABLE 1

Family 56.6 from F_2 55.105.8 (normal plant) × 54.27G1 (flaked R_2 plant)

Plant number	Plant type	Pollen* type	Chromosome type	Fruitfulness†
56.6.1	flaked	+ or ±	12 pairs + 1 fragment	—
56.6.2	flaked	+ or ±	12 pairs + 1 whole + 1 fragment or trivalent	±
56.6.3	normal	+ or ±	12 pairs (1 tetravalent)	+
56.6.4	flaked	±	12 pairs + 1 whole + 1 fragment	—
56.6.5	flaked	+	12 pairs + 1 fragment	1 fruit
56.6.6	mutant	+	12 pairs (1 weakly joined pair)	±
56.6.7	mutant	0	12 pairs + 1 fragment (1 tetravalent)	—
56.6.8	normal	0	12 pairs + 1 whole	—
56.6.9	flaked	0	12 pairs + 1 fragment	1 fruit
56.6.10	flaked	±	12 pairs + 1 trivalent + 1 fragment	1 fruit
56.6.11	flaked	0	12 pairs + 1 fragment (1 tetravalent)	—

* Pollen scale: 50 percent stainable (+), 25 percent stainable (±), 0 percent (0).
† Fruitfulness: good (+), fair (±), poor (—).

1 that all flaked plants in this family had 12 pairs of chromosomes and a small fragment and that tetravalence may occur in either flaked or nonflaked plants.

Another family (56.98) similar in origin to 56.6, from a nonflaked parent selfed, gave two nonflaked and three flaked plants. The flaked plants had 12 pairs and a small fragment.

The discussion of flaked which follows refers to plants obtained by selfing or by crossing with nonflaked lines. These plants were free from multivalence and no longer produced either albino or wiry mutants.

Flaked plants always have 12 pairs and one or two small fragments in some of their p.m.c. The glabrous sterile shoots also had this chromosome complement so far as tested. Since the fragment is so small (Figures 5 and 6) and tends to be weakly stained, it is impossible to be sure that it is not present in cells in which it cannot be seen. It is most easily seen at lateral M1 since it was often above or below the metaphase alignment. It has also been seen lagging or dividing belatedly at A1 and M2. It is sometimes attached to a pair of chromosomes at diakinesis and M1. When one fragment is present it is often lost in meiosis; when two fragments are present they are often weakly paired but one of them may be attached to the pair referred to above, the other free. As far as could be ascer-

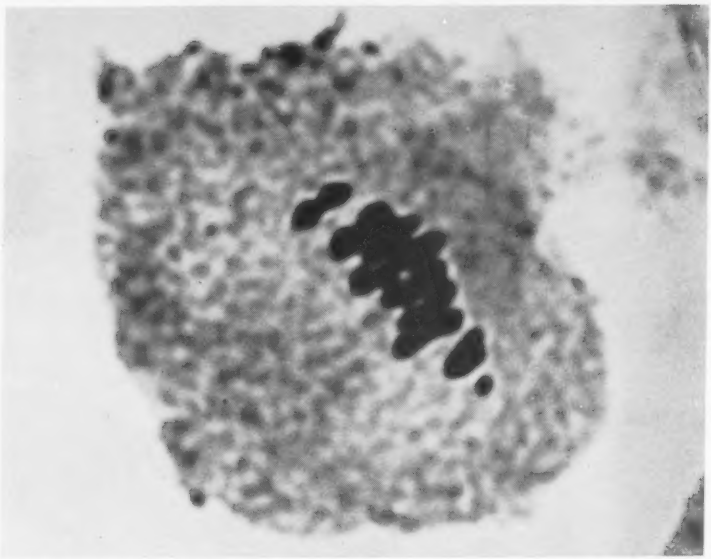

FIGURE 5.—First metaphase in p.m.c. of flaked. Fragment is exceptionally deeply stained.

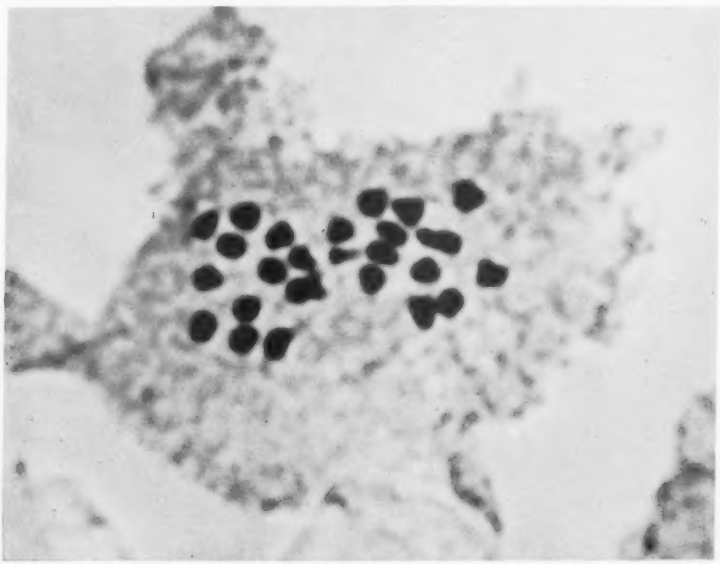

FIGURE 6.—Second metaphase in p.m.c. of flaked; undivided fragment going belatedly to one plate.

tained the size of the fragment has remained very much the same for eight generations of transmission. One pair of chromosomes is often weakly associated; this may result from competition for pairing. It might also indicate an injured centromere region or a deficiency in one or both homologues.

In F_1, 12 pairs of chromosomes and a fragment are usually present but in one plant no fragment was found. Since F_1 plants always appear normal, it is assumed that flaked plants contain a pair of deficient chromosomes and a fragment. A deficient chromosome and a fragment are complementary, making production of normal-looking tissue possible. In F_1 the presence of one unaltered chromosome results in a normal-looking plant. Plants containing two deficient chromosomes but lacking a fragment are nonviable.

At pachytene the fragment has been seen attached by one end near the centromere region of chromosome 6 (BARTON 1950). This chromosome has three large heterochromatic regions (Figure 7) and can therefore be identified in many

7

FIGURE 7.—Pachytene in p.m.c. of flaked. The fragment is attached near the centromere of chromosome 6.

8

FIGURE 8.—Mitosis in tapetal cells of flaked. The undivided fragment lags. In one plate the central chromosomes were not countable.

cells. The fragment is rod-shaped with two small darker-staining regions. In F_1 pairing seems normal in chromosome 6 except for a possible nonconformity within the heterochromatic portion of the short arm.

In tapetal cells the fragment has been seen to lag (Figure 8). The lagging of the fragment in mitosis accounts for the somatic instability of flaked and for the probable absence of the fragment in some p.m.c. of F_1 plants.

Genetics of flaked: Flaked is self-fertile. Eleven families containing 231 plants which originated from controlled self-pollination of flaked parents were all flaked. F_1 flaked × nonflaked was nonflaked in ten families and the reciprocal cross gave eight families of nonflaked. Flaked was completely recessive. The F_2 data (Table 2) from crosses of flaked with unrelated lines containing various recessive genes seem to be conflicting. Flaked plants were always in a minority and were entirely absent in nine families. In 56.163.1 the observed ratio 186:0 differs very significantly from 15:1, with $x^2 = 12$, $P < 0.01$. In all F_2 families combined, the nonflaked:flaked ratio is 1673:161 and fits neither a 3:1 nor a 15:1 ratio. With a 3:1 expectation the heterogeneity is $x^2 = 58$, D.F. = 19 and $P < 0.01$, indicating that the F_2 deviations are not due to random sampling.

An arbitrary separation of the F_2 families containing flaked from those not containing it was made. In the 11 families containing flaked the ratio is 998:161 or 6.2:1 so that flaked was transmitted to about one seventh of the F_2 generation. By the method of BRAND and SNEDECOR quoted by MATHER (1938), with the expected ratio 6.2:1, the heterogeneity $x^2 = 18.5$, with D.F. = 10, and $P = 0.05$, is not significant. With a 3:1 expectation the deviation x^2 is 76, D.F. = 10 and $P < 0.01$ and with 15:1, the deviation is even greater.

TABLE 2

F_2 from flaked × nonflaked and the reciprocal cross

F_1 parents*	P_1	F_2		Seed germination (percent)
		Nonflaked	Flaked	
55.24.10	Flaked × nonflaked	5	3	45
55.24.11	Flaked × nonflaked	22	1	85
57.103.1	Flaked × nonflaked	360	55	85
58.181.4	Flaked × nonflaked	261	56	99
59.092.1	Flaked × nonflaked	53	7	94
59.091.4	Flaked × nonflaked	55	8	97
60.030.1	Flaked × nonflaked	51	0	89
56.137.1	Flaked × nonflaked	30	0	91
56.163.1	Flaked × nonflaked	186	0	97
59.131.1	Flaked × nonflaked	64	0	100
59.131.2	Flaked × nonflaked	56	0	88
60.069.5	Flaked × nonflaked	51	6	86
60.025.1	Flaked × nonflaked	50	3	83
57.102.1	Nonflaked × flaked	77	16	98
59.129.1	Nonflaked × flaked	56	5	95
59.136.1	Nonflaked × flaked	10	1	35
56.139.1	Nonflaked × flaked	55	0	86
59.060.2	Nonflaked × flaked	53	0	83
59.134.1	Nonflaked × flaked	72	0	75
59.130.1	Nonflaked × flaked	62	0	97

* All F_1 parents were nonflaked.

No significant difference was found in F_2 from reciprocal crosses. Flaked plants occurred in three families from F_1 nonflaked × flaked. This evidence indicates that inheritance is not cytoplasmic. In F_2 families 58.181.4 and 59.091.4 seed germination was high but only 17 percent were flaked; in F_2 families 56.163.1 and 59.131.1 (Table 2) originating from flaked × nonflaked and the reciprocal no flaked occurred. The deficiency of flaked was not due to low seed viability.

The genetic evidence is consistent with the theory that flaked contains a pair of deficient chromosomes and one or more fragments. Controlled self-pollination of flaked gave only flaked progeny. F_1 plants were all nonflaked. All flaked plants contain one or more fragments and are somatically unstable. The sporocytes of F_1 plants contain a normal chromosome, a deficient chromosome, and may or may not contain a fragment. Only those containing a fragment will transmit flaked and probably only gametes containing a normal chromosome or a deficient chromosome and a fragment are viable. The proportion of flaked in F_2 will depend on the presence or absence of the fragment in the sporocytes. Figure 9 shows that from sporocytes containing one fragment the expected F_2 ratio, nonflaked:flaked, is 8:1. The observed ratios from parents 59.092.1 and 59.091.4 (Table 2) approach this but other ratios deviate from it in both directions. The proportion of flaked is expected to be higher if one or both sporocytes contained two fragments and it

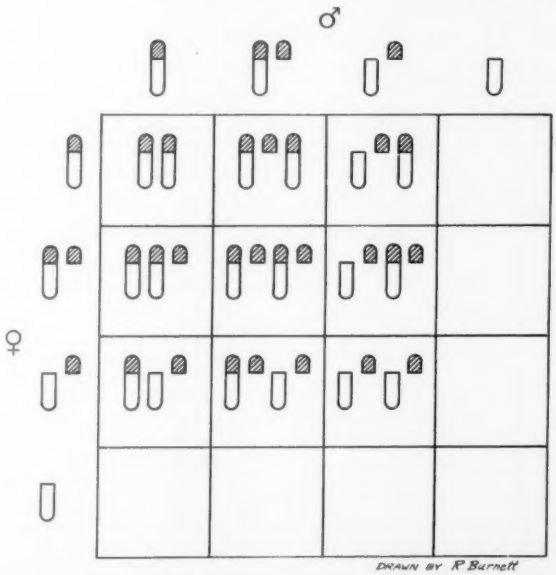

FIGURE 9.—The possible combinations of gametes of F_1 normal × flaked having one normal, one deficient, and one fragment chromosome, if only gametes containing a whole chromosome or a deficient chromosome and a chromosome fragment are viable.

Fragment is shaded, chromosome lacking shaded portion is deficient.

would be lowered by loss of the fragment, which is known to occur in meiosis or if the sporocytes lacked the fragment. The karyotype (Figure 9) containing two normal chromosomes and one or two additional fragments has not been identified. It would not be flaked and would be trisomic for a portion of chromosome 6.

DISCUSSION

Pachytene study suggests that the deficient chromosome and fragment of flaked are components of chromosome 6, which contains linkage group IV. There is, in this chromosome, a recessive gene *md* (*mo*) which originated from thermal neutron radiation. The gene *md* causes small white spots on the leaves, and, in this respect, seems to be similar to flaked. Unlike flaked *md* is temperature sensitive (BURDICK 1959) and has normal gametic and zygotic viability. Mendelian inheritance of flaked is certainly not indicated on any conventional basis. Moreover, in flaked plants variegation in chlorophyll and in hairiness are always associated. Lack of hairs can occur without white flaking in unrelated material but this is not true in the flaked line. It therefore seems probable that in flaked the fragment contains two loci which are seldom, if ever, separated. The original line in which flaked occurred did not contain the recessive alleles *yg2*, *yg3* and *md*. It is possible that a mutation similar to *md* occurred when the fragment was broken off.

The variegation in chlorophyll and in hairiness may be due simply to the different effect of the presence or absence of the fragment in subepidermal and epidermal layers. If the fragment is present, the epidermis is hairy, if absent, it is glabrous. In the subepidermal layer, chlorophyll and palisade cells are normal if the fragment is present, but, if absent, no chlorophyll or palisade cells are found. Many geneticists believe and cytological observations confirm that the chromosome content of these layers is normally the same. If so, the deficient chromosome has a very different effect in different cell layers. Presumably different loci are active in the epidermal and subepidermal layers.

The observed variation in the degree of flaking in different plants is explained by variation in frequency of somatic fragment loss. It might also be due to breakage of the fragment and consequent gene loss.

That telocentric fragments tend toward loss in the soma and misdivision, as well as toward union to form isochromosomes, was demonstrated by DARLINGTON (1939). RHOADES (1940) and SEARS (1952 a, b) showed that similar behavior of telocentric fragments results in variegation in rye and in wheat. The centromere of the fragment in flaked is at or near one end. It is possible that neither the altered chromosome 6 nor the fragment has a complete centromere. It was noted above that one pair of chromosomes in flaked is often more or less separate at M1.

In chromosome 6, a large heterochromatic region lies on each side of the centromere, and the fragment must have been derived from one of these. In F_1, the only observed difference in pachytene pairing appears to be within the heterochromatic mass in the short arm, and the fragment has been found attached to chromosome 6 near this region. Changes in position and amount of

heterochromatin have been adduced as inducing variegation (LEWIS 1950; MCCLINTOCK 1950).

The fragment in flaked is very hard to find in some smears. It tends to be much paler than the other chromosomes and, except in very well stained material, could easily be missed. It seems conceivable that fragments too small to be demonstrated with certainty may cause variegation.

SUMMARY

"Flaked," a variegated tomato plant, occurred in R_2 from a seed soaked in a solution of P^{32} as phosphate. Normal green areas are irregularly variegated with small whitish flakes in which the palisade cells are replaced by nongreen parenchyma. Variegation due to loss of hairs also occurs but it is distributed independently and may give rise to glabrous shoots.

Flaked has rather poor pollen and produces few fruits, which vary greatly in seed production. Selfed, it produced only flaked. All flaked plants have either one or two small fragments in addition to 12 pairs of chromosomes. The fragment tends to be lost both in meiosis and mitosis.

All F_1 plants look normal; usually they have 12 pairs of chromosomes and one fragment but occasional F_1 plants lack the fragment.

At pachytene the fragment is a single strand and is sometimes found attached to chromosome 6. Recessive genes affecting chlorophyll are known to be present in this chromosome but the line in which flaked occurred was homozygous for the normal alleles.

Since all flaked plants have the fragment and all F_1 plants from crossing non-flaked and flaked are normal, it follows that flaked plants lack a normal chromosome 6. The normal parts of flaked plants contain a fragment in addition to the deficient chromosome. The white flakes are believed to lack the fragment and are therefore deficient. The partial male sterility of flaked suggests that only gametes containing the fragment in addition to a deficient chromosome are viable. In the epidermis the loss of the fragment results in loss of hairs. Buds from glabrous shoots may have 12 pairs of chromosomes and one fragment. They are always sterile.

Loss of the fragment in the soma of an F_1 plant would give rise to germ cells that lack it, but would not affect the appearance of the plant since all cells contain a normal chromosome 6. F_2 families contained normal and flaked plants in the mean ratio of six normal to one flaked. Several F_2 families, however, contained no flaked plants. Neither a 3:1 nor a 15:1 ratio is indicated. It is believed that loss of the fragment in mitosis may explain the irregular transmission of flaked.

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The newly formed American Society for Cell Biology has announced its first regular meeting and has issued a call for papers. The meeting will be held November 2, 3, and 4, 1961, in Chicago, Illinois at the Edgewater Beach Hotel. A program of three symposia, contributed papers, demonstrations and motion pictures is being arranged. The symposia with invited speakers from the U. S. and abroad, reflect the Society's aim to transcend disciplinary boundaries and bring together workers with divergent approaches in an effort to focus on fundamental problems of the cell. The topics will cover the following:

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III. Characteristics of cell interfaces: The chemistry and the fine structure of cell membrane systems, and problems of transport across membranes.

All interested cell biologists, whether they are members or not, are invited to submit papers (15 minute presentation time including discussion) for the contributed papers sessions. Demonstration papers and motion pictures are also solicited. Abstracts of 300 words or less are due to be in the hands of the Chairman of the Program Committee, Dr. Hewson Swift, Department of Zoology, University of Chicago, Chicago 37, Illinois, *before* August 15, 1961. The Program Committee includes the following:

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